RESPONSE OF SOYBEAN TO THE INTERACTIONS AMONG THREE ROOT INFECTING FUNGI, A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS AND *RHIZOBIUM JAPONICUM*

BY

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TO MY PARENTS,
MY WIFE, EUNIZE,
AND
MY DAUGHTER, LARISSA

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RESPONSE OF SOYBEAN TO THE INTERACTIONS AMONG THREE ROOT INFECTING FUNGI, A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS AND *RHIZOBIUM JAPONICUM*

By

Laércio Zambolim

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Chairman: N. C. Schenck
Major Department: Plant Pathology

Interactions among *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and two symbiotic organisms, *Glomus mosseae* and *Rhizobium japonicum*, and their effect on the severity of soybean root and stem rot were investigated by exposing nodulated and non-nodulated seedlings to defined inoculum densities of the various organisms in greenhouse tests. Autoclaved or non-autoclaved Arredondo fine sand with 91 ppm of phosphorus and pH 6.7 was thoroughly mixed with spores or resting structures of the fungi. The mycorrhizal fungus was added at 500 chlamydospores per pot in one layer 5 cm below the seeds. *Rhizobium japonicum* cells were inoculated directly on the seeds at 5 mg/g of seed. The percentage of infected roots, diseased plants, or both increased with increasing levels of inoculum of each fungus. The concentrations at which 50% of the roots were infected (ED50) by *M. phaseolina* were approximately 20 x 10^3 and 150 x 10^3 sclerotia per kilogram of autoclaved and non-autoclaved soil, respectively. For

R. solani the ED50s were 1.0 x 10^3 and 2.0 x 10^3 sclerotia per kilogram of autoclaved and non-autoclaved soil, respectively.

Macrophomina phaseolina at 40 x 10^3 sclerotia per kilogram of soil interacted additively with R. solani at 1.0 x 10^3 sclerotia per kilogram of soil and with F. solani at 3,000 x 10^3 chlamydospores per kilogram of soil to increase root and stem rot and to reduce the plant growth response. Root and shoot weight and plant height were reduced more at 45 than at 25 days after planting, and soybean seed yield was reduced 20-30% in autoclaved and 10-16% in non-autoclaved field soil by exposure to the pathogens. The number and weight of nodules were reduced greatly by each of the three pathogens.

In the presence of the mycorrhizal fungus, G. mosseae, plant growth responses, nodule numbers and weight, and seed yield of plants infected with M. phaseolina, R. solani and F. solani were equal to or superior to the uninoculated plants. The mean seed yield in three experiments with the variety 'Hood' was increased 49% in autoclaved and 17% in non-autoclaved field soil by G. mosseae. The populations of the three pathogens and disease severity were not reduced significantly by G. mosseae, but the colonization of roots by G. mosseae was reduced significantly by the pathogens.

Growth responses of plants exposed to G. mosseae were always significantly better than those of the nonmycorrhizal controls for both nodulated and non-nodulated "Hardee" soybeans.

Total nutrients in the shoots were significantly increased in plants infected with G. mosseae but were reduced in those infected with M. phaseolina.

GENERAL INTRODUCTION

Soybean (Glycine max (L.) Merrill) is one of the most widely cultivated plants and is believed to be a native of the eastern half of north China, where it was first domesticated about the 11th century B.C. (62). Because soybean is a source of relatively inexpensive vegetable oil and high quality protein, there has been a sharp increase in soybean acreage. In the United States, soybean expanded from 43 million acres in 1971 to 58 million in 1977, which is a 33% increase. Soybean acreage increased 61% during this time in Florida. Current trends in soybean production in Florida are expected to continue, and rates of increase in acreage planted to this crop are expected to accelerate. With this increase in soybean acreage there may also be a concurrent increase in the number and severity of soybean diseases (105), especially those caused by soil-borne pathogens (118).

Several reports in the literature indicated that soil-borne diseases caused by fungi on soybean appear complex and can result in crop losses. In samples of soybean rhizosphere soil from 40 locations in north Florida, Schenck and Kinloch (109) found Fusarium spp., Rhizoctonia spp., and Macrophomina phaseolina present in a great majority of samples, but they also found spores of vesicular-arbuscular mycorrhizal fungi in every sample. Furthermore, Schenck (106) demonstrated that the suppression of soil-borne pathogens with sodium azide

increased soybean yields. In addition to the damage that these pathogens may cause individually, they also may interact synergistically with other pathogens and increase disease severity. According to Powell (96), indirect losses caused by interaction of nematodes and soil-borne fungi on soybean may cause greater losses than nematodes alone.

Despite the potential importance of a complex of root infecting fungi on soybean, there is little information in the literature on the effect of fungal complexes or associated symbiotic organisms in the rhizosphere of soybean on plant production. The study of interactions among rhizosphere organisms would be of great value in integrated pest management programs in which effects of symbiotic organisms on plant pathogens are evaluated. Symbiotic organisms possibly could be used to reduce the action or effects of plant pathogens and thus result in less expenditures of energy, time, and money in a disease control program.

SECTION I

EFFECT OF MACROPHOMINA PHASEOLINA ALONE OR IN COMBINATION WITH RHIZOCTONIA SOLANI OR FUSARIUM SOLANI ON SOYBEAN STEM AND ROOT ROT

Introduction

Disease complexes are formed in nature when plants are attacked simultaneously by more than one pathogenic organism. Under such conditions distinct biological changes can occur in both the host and the pathogenic organisms involved. Plants under attack by one pathogenic organism may become more or less susceptible to additional pathogens, or the presence of one pathogen may inhibit or stimulate the growth of a second pathogen.

Interactions between soil-borne plant pathogenic fungi have been demonstrated to greatly influence disease incidence and severity on many crops. Some interactions among plant pathogenic fungi resulted in synergism (43, 44, 93) while others resulted in antagonism (61, 72, 87, 93).

Several important pathogens have been reported to cause root and stem rot on soybean (16, 40, 75, 117, 126), but most reports deal primarily with damage caused by individual pathogens. The parasitism of Rhizoctonia solani Kühn on soybean was first studied by Boosalis (12). Diseases caused by Rhizoctonia solani, such as root rot, stem rot, damping-off, and aerial blight, have been reported from all

soybean growing areas of the world (1, 39, 56, 75, 120, 126). Stand losses of soybean of up to 50%, and yield losses in the field of up to 40% have been reported in the United States (126) and Brazil (75), respectively. In Florida, R. solani is prevalent throughout the state causing serious losses on a variety of crops each year, and is perhaps the most important soil-borne plant pathogen (105). In Minessota, R. solani root rot is considered a very destructive disease on soybean (12).

Fusarium spp. have been reported in association with soybean root and stem diseases since 1917, when Cromwell (22) reported a blight of soybean caused by Fusarium tracheiphilum. Several species of Fusarium, including F. tracheiphilum Armst. & Armst., F. vasinfectum Amst. & Amst., F. orthoceras (F. oxysporum Snyd. & Hans.) (40) and F. solani (Mart.) App. & Wr. emend. Snyd. & Hans. (16) have been reported to cause diseases on soybean. Fusarium root rot, referred to as Fusarium blight of soybean (37), was first reported to be a problem in Iowa in 1953 (35). Warren and Kommedahl (133) reported that five Fusarium spp. were isolated from soybean roots, rhizospheres, plant residues and soil; 16-33% of the isolates were F. solani. A survey of soybean diseases in Iowa in 1955 revealed that 95% of the fields had plants infected by Fusarium spp. (36). Fusarium species are also prevalent in Florida soils and cause root rot, damping-off, and wilt of several crops (105). In surveys of soybean roots and rhizosphere soil from 40 locations in Northern Florida in 1971 and 1972, Fusarium spp. were the most common pathogenic fungi isolated (109).

Charcoal rot caused by Macrophomina phaseolina (Tassi.) Goid, occurs throughout the world and can be a destructive pathogen on a

wide variety of plants, especially in hot dry weather (118, 140, 142) or when plant growth is limited by unfavorable environmental conditions (118). The disease is more evident at the end of the growing season (117), but field plants may be affected at any age (63). Considerable losses due to charcoal rot have been reported in the United States (117), and losses up to 50% and 70% have been recorded in Yugoslavia (2) and India (41), respectively.

Although M. phaseolina, R. solani, and F. solani were reported causing losses individually, they might be involved in disease complexes resulting in synergistic disease increases. Macrophomina phaseolina has been reported as increasing the severity of root rot caused by Fusarium spp. (60, 78, 97, 116), Rhizoctonia solani (116, 131), Verticillium sp. (137), and Neocosmospora vasinfecta E. F. Smith (13). However, no information is available on the effect of F. solani or R. solani on M. phaseolina in the rhizosphere of soybean.

Our preliminary research results, field observations, and literature information suggested that there were interactions among these soybean root rot pathogens in disease development. Therefore, the purpose of this study was to determine the effect of M. phaseolina alone and in combination with R. solani or F. solani, on the severity of stem and root rot of soybean.

Materials and Methods

The fungi used in this study were isolated from field infected soybean plants. Hyphal-tipped isolates of M. phaseolina, R. solani, and F. solani were grown on Difco potato dextrose agar (PDA) at 27 C and were maintained as stock cultures at 5 C on PDA in test tube slants.

Isolate no. 3 of M. phaseolina (Table 1) used in this investigation was selected for its ability to produce the largest lesion on wounded inoculated soybean seedling hypocotyls. The isolates of R. solani and F. solani selected were those that resulted in the greatest number of infected plants growing in autoclaved soil infested with 10 ml of a 5-day old mycelial suspension placed in a single layer 2 cm below the seeds. Ten 15-cm pots containing five plants each were used for each isolate.

Arredondo fine sand from a soybean field at the Agronomy Farm, University of Florida, was used throughout this study after it was autoclaved twice at 12 pounds per square inch and 121 C for 4 hr at 24 hr intervals. Soil analysis by the University of Florida Soil Science Department indicated a soil pH of 6.4 and nutrient contents of 93 ppm P, 52 ppm K, 325 ppm Ca, 84 ppm Mg, 38 ppm NO_3. 384 ppm Al, 11.6 ppm Fe, 1.96 ppm Zn. Soil extraction of phosphorus was accomplished by the double acid extracting solution method (0.05 N hydrochloric acid plus 0.025 N sulfuric acid). Soil pH was determined using a 1:2 soil: water (wt:vol.) ratio and a standard pH meter.

Sclerotia of M. phaseolina were produced by placing four 4-mm-diameter PDA disks from a 72-hr-old PDA culture into a 1,000 ml Erlenmeyer flask containing 100 ml of soybean seed extract broth. The broth was prepared by boiling 100 g of dry soybean seeds in 500 ml of distilled water for 10-15 min, filtering the extract through six layers of cotton gauze, adjusting the filtrate volume to 1 liter with distilled water, adding 20 g of sucrose, and then autoclaving (121 C for 15 min). After 15 days, the mycelial mats with sclerotia were homogenized in a

vortex mixer with 20 ml of sterile distilled water to break up the mat and separate the sclerotia from the mycelium. The homogenate was filtered through Whatman no. 42 filter paper, washed with sterile distilled water three to four times, and dried for 48 hr. Clusters of sclerotia were separated by grinding in a mortar, and individual sclerotia were separated by passage through a 149-µm sieve. Sclerotia stored dry in vials at 5 C remained viable for more than 1 yr. After rinsing in sterile water, the viability of surface sterilized (0.5% NaClO for 60 sec) sclerotia was tested on PDA supplemented with 30 µg/ml streptomycin sulfate. Sclerotia that had been stored in vials were added to approximately 500 ml of water and sclerotial numbers were estimated by counting ten 1-ml samples.

Chlamydospores of F. solani were obtained by placing two 4-mm diameter PDA disks from a 5-day-old PDA culture into a 250-ml Erlenmeyer flask containing 50 ml of soil extract. Soil extract was prepared by autoclaving 500 g of field soil in 1,000 ml of tap water for 30 min, filtering the extract through Whatman no. 10 filter paper, adjusting the pH to 6.5 with $CaCO_3$, and then autoclaving at 121 C for 15 min. After incubation for 7-10 days at 27 C, the contents of several flasks were decanted onto a 44-µm sieve and the mycelial mats with chlamydospores were gently rinsed. The mats were ground in a Pyrex tissue grinder containing 10 ml of deionized water until no mycelia were evident. After grinding, the volume of the homogenate was increased with deionized water and 80 ml amounts were sonicated with a Biosonic III Ultrasonic system at 40% of maximum power for 40 sec.

Table 1. Sources of isolates of Macrophomina phaseolina and average length of necrotic lesions produced by them on wounded hypocotyls of 30-day old soybean seedlings in a growth chamber at 30 C.

Isolate Source	Isolate	Average length of the necrotic lesions (mm) 20 days after inoculation*
Soybean root	1	16
Soybean root	2	25
Soybean root	3	26
Soybean root	4	16
Soybean field soil	5	20
Soybean field soil	6	16
Soybean stem	7	16
Soybean stem	8	24
Soybean stem	9	23
Soybean stem	10	14
University of Missouri	11	16

* The experiment was repeated three times; values represent the mean of 30 soybean seedlings inoculated with 5-mm diameter plugs from the edge of a 7-day old culture.

After sonication, the number of chlamydospores/ml was determined with a hemacytometer.

Sclerotia of R. solani were produced by placing four 4-mm diameter PDA disks from a 2-day-old PDA culture into a 250-ml Erlenmeyer flask containing 50 g of mixture of corn meal and sand (45 g of double washed sand plus 5 g corn meal plus 15 ml of distilled water). After incubation for 30 days at 27 C, the contents of several flasks were blended in sterile tap water for 60 sec at maximum intensity in a blender, and the resulting suspension was passed through nested 125 and 250-µm sieves. The mycelial fragments were removed by exposure to a high pressure water spray. After resuspending the sclerotia in 1,000 ml of tap water, the sclerotial numbers were estimated by counting ten 1-ml samples.

Soybean seeds (Glycine max var. Hood), which were surface disinfested with 1.0% sodium hypochlorite for 60 sec and rinsed three times with sterile water, were used in all experiments.

For the quantitative population estimates of the organisms, the infested soil was plated on selective media. The number of propagules of M. phaseolina per gram of soil was determined by the technique described by Papavizas and Klag (92). Ten grams of air dried soil were suspended in 50 ml of distilled water for 60 sec, and the suspension was passed through 250- and 88-µm sieves. The contents of the 88-µm sieve were exposed to a 0.5% solution of sodium hypochlorite for 8 min before aliquots were pipetted from the final dilution onto the surface of the selective medium. After incubation in the dark at 30 C for 12 days, the colonies of M. phaseolina were counted. Rhizoctonia solani populations were estimated according to the method described by Ko and

Hora (71). Ten grams of air dried soil were mixed on a 5 x 10 x 0.5 cm tray with 10 ml of 2.5% water agar containing 50 mg of streptomycin sulfate/liter of medium. The solidified agar-soil sample was divided into 100 squares and 10 squares were evenly distributed on each of 10 plates of selective medium. After incubation in the dark at 30 C for 36 hr, the colonies of R. solani were counted. The number of propagules of F. solani per gram of air-dried soil was determined using a dilution plate method with a selective medium containing pentachloronitrobenzene (PCNB) (85). One gram of soil was suspended in 300 ml of 0.1% water-agar containing 250 mg of streptomycin sulfate per liter of medium. A 1-ml aliquot was pipetted onto each of 10 plates and was spread over the surface of the agar with a bent glass rod. Colony counts were taken after plates had been incubated for 10 days at room temperature.

For the evaluation of root infection in all experiments, 20 2.5-cm soybean root pieces were taken at random from each of 30 plants, in treatments in which soil was infested with M. phaseolina or F. solani or from 30 to 40 hypocotyls from plants in treatments in which soil was infested with R. solani. Root pieces were surface sterilized in a 0.5% solution of sodium hypochlorite and plated on 2% water-agar plates which were maintained at 27 C. After 12 days of incubation, the percentages of root infection were assessed by determining the number of roots from which colonies of M. phaseolina or F. solani originated. Soybean hypocotyls with typical mycelia of R. solani were evaluated 36 hr after incubation.

For the inoculum density studies, soil was mixed thoroughly for 5 min with an electric Hobbart mixer with the following inoculum levels:

0.0, 2.5, 5.0, 7.5, 10.0, 20.0, 40.0 and 80.0 sclerotia of M. phaseolina per gram of autoclaved soil, or 0.0, 25.0, 50.0, 75.0, 100.0, 150.0, 250.0, and 500.0 sclerotia per gram of field soil; 0.0, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0 and 8.0 sclerotia of R. solani per gram of either autoclaved or field soil; 250, 375, 750, 1,500, 2,000 and 3,000 chlamydospores of F. solani per gram of autoclaved soil. Water was added to the soil during the mixing process to give a final water content of 10% (W/W). The experiments were conducted in a greenhouse at 28-35 C in thirty 10-cm clay pots containing 500 g of soil with 2 soybean plants per pot per treatment. Twenty-five days after seeding, soybean plants were harvested to evaluate the percentage of root and stem infection.

An experiment was conducted to evaluate the interactions of M. phaseolina on F. solani and R. solani. Autoclaved soil was infested with 20 and 40 sclerotia of M. phaseolina, 1 sclerotium of R. solani and 250 and 3,000 chlamydospores of F. solani per gram of soil. Soil was mixed thoroughly with an electric Hobbart mixer, and water was added to the soil to give a final water content of 10% (W/W). Two seeds were planted in 2,000 g of soil in each of 40 15-cm clay pots per treatment and the pots were maintained in a greenhouse at 28-35 C. Twenty-five days after seeding, soybean plants were harvested to evaluate percentage of root and stem infection, plant height, and root and shoot weights; soil samples were plated to determine the populations of the pathogens.

Results

Relationship of inoculum density to disease severity

The percentage of soybean roots and seedlings infected with M. phaseolina, the percentage of diseased plants with R. solani, and the percentage of roots infected with F. solani increased with increased levels of inoculum of each fungus added to either autoclaved or non-autoclaved soils.

After 25 days, 100% of the seedlings were infected at 20 x 10^3 sclerotia of M. phaseolina per kilogram of autoclaved and non-autoclaved soil (Fig. 1). When the infection data were transformed to Log_e 1/(1-x), where x equals the proportion of the diseased seedlings to adjust for multiple infection (130), and plotted against Log_{10} of the number of sclerotia of M. phaseolina per kilogram of soil (Fig. 2), the slope of the line determined by linear regression analysis was 1.69 for autoclaved and 0.97 for non-autoclaved soil. The inoculum density required for 50% infection of the plants was interpolated to be approximately 8.0 and 38 x 10^3 sclerotia per kilogram of autoclaved and non-autoclaved soil, respectively.

When soybean roots were plated on 2% water-agar, 50% of the root pieces were infected at approximately 20 x 10^3 or 150 x 10^3 sclerotia of M. phaseolina per kilogram of autoclaved or non-autoclaved soil, respectively (Fig. 3). The maximum proportion of infected roots obtained was 84% at approximately 80 x 10^3 sclerotia per kilogram of autoclaved soil, and 85% at 500 x 10^3 sclerotia per kilogram of non-autoclaved soil after 25 days. The ED50 of soybean roots infected by M. phaseolina in autoclaved soil was approximately 20 x 10^3 and

Fig. 1. The relationship of density of sclerotia of Macrophomina phaseolina in autoclaved (o———o) and non-autoclaved (o------o) soil to percentage of soybean seedlings infected 25 days after planting.

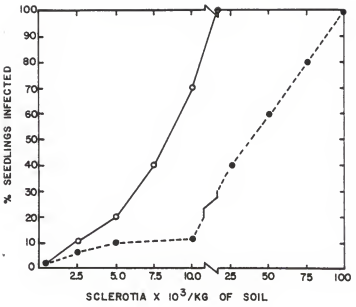

% SEEDLINGS INFECTED
100
90
80
70
60
50
40
30
20
10
0
2.5
5.0
7.5
10.0
25
50
75
100
SCLEROTIA X 10^3/KG OF SOIL

Fig. 2. The relationship of density of sclerotia (Log_{10}) of Macrophomina phaseolina in autoclaved (o———o) and non-autoclaved (o------o) soil to percentage of infected soybean seedlings ($Log_{10}[Log_e\ 1/(1-x)]$), where x = proportion of infected seedlings 25 days after planting.

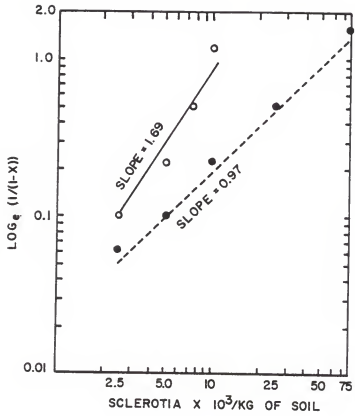

2.0
1.0
0.1
0.01
LOG$_e$ (1/(1-X))
SLOPE = 1.69
SLOPE = 0.97
2.5
5.0
10
25
50
75
SCLEROTIA X 10^3/KG OF SOIL

Fig. 3. The relationship of density of sclerotia of Macrophomina phaseolina in autoclaved (o———o) and non-autoclaved (o------o) soil to percentage of soybean roots infected 25 days after planting.

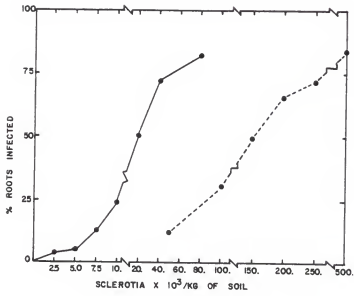
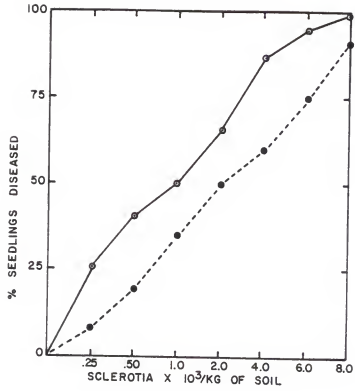

100
75
50
25
0
% ROOTS INFECTED
2.5 5.0 7.5 10. 20. 40. 60. 80. 100. 150. 200. 250. 500.
SCLEROTIA X 10^3/KG OF SOIL

Fig. 4. The relationship of density of sclerotia of Rhizoctonia solani in autoclaved (o———o) and non-autoclaved (o------o) soil to percentage of diseased soybean seedlings 25 days after planting.


100
75
50
25
0
% SEEDLINGS DISEASED
.25
.50
1.0
2.0
4.0
6.0
8.0
SCLEROTIA X 10³/KG OF SOIL

150 x 10^3 sclerotia per kilogram of autoclaved and non-autoclaved soil, respectively.

For R. solani, 8.0 x 10^3 sclerotia per kilogram of soil, the highest level of inoculum used in the experiment, resulted in 100 and 92% infection of plants in autoclaved and non-autoclaved soil, respectively (Fig. 4). The slope of the line was determined to be 0.64 for autoclaved and 0.87 for non-autoclaved soil (Fig. 5). The inoculum level required for 50% infection of the plants was interpolated to be 1.0 and 2.0 x 10^3 sclerotia per kilogram of autoclaved and non-autoclaved soil, respectively.

After 25 days, only 43% of the soybean roots exposed to F. solani were infected at 3 x 10^6 chlamydospores per kilogram of soil (Fig. 6).

Without exception, plants infected with M. phaseolina and F. solani showed no symptoms at 25 days after planting, except for reduction in plant size. Plants grown in soil infested with R. solani had lesions on the hypocotyl, and those exposed to more than 8.0 x 10^3 sclerotia per kilogram of soil died within 25 days after planting. Similar results were obtained when these experiments were repeated.

Soybean response to the interaction between Macrophomina phaseolina and Fusarium solani

After 25 days, no significant differences occurred among the treatments for all traits analyzed (Table 2). At high levels of inoculum, M. phaseolina significantly reduced root weight by 27%, but only slightly reduced shoot weight (10%) and plant height (11%) as compared to non-inoculated control plants (Table 3). Fusarium solani significantly reduced root weight (41%), shoot weight (42%) and plant height

Fig. 5. The relationship of density of sclerotia (Log_{10}) of Rhizoctonia solani in autoclaved (o———o) and non-autoclaved (o-------o) soil to percentage of diseased soybean seedlings ($Log_{10}[Log_e 1/(1-x)]$), where x = proportion of diseased seedlings 25 days after planting.

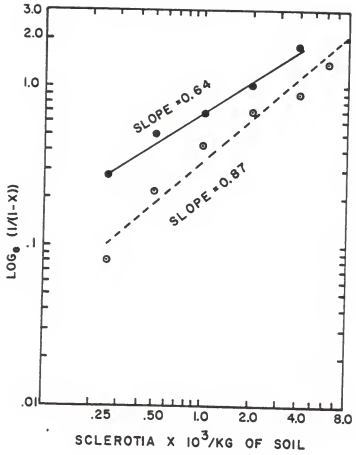

3.0
2.0
1.0
.1
.01
SLOPE = 0.64
SLOPE = 0.87
LOG_e (1/(1-X))
.25
.50
1.0
2.0
4.0
8.0
SCLEROTIA X 10^3/KG OF SOIL

Fig. 6. The relationship of density of chlamydospores of Fusarium solani in autoclaved soil (o———o) to percentage of soybean roots infected 25 days after planting.

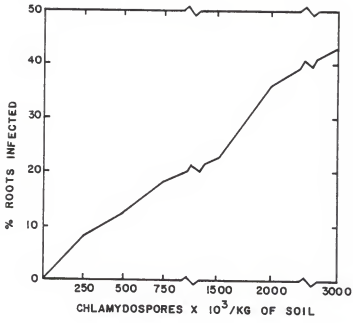

% ROOTS INFECTED
0
10
20
30
40
50
250
500
750
1500
2000
3000
CHLAMYDOSPORES X 10^3/KG OF SOIL

Table 2. Soybean response at 25 and 45 days, and percentage of roots infected 45 days after infesting soil with known quantities of Macrophomina phaseolina and Fusarium solani alone or in combination.[y]

Treatments	Root weight (g)		Shoot weight (g)		Plant height (cm)		Roots with M. phaseolina (%)	Roots with F. solani (%)
	25	45	25	45	25	45	45	45
Non-inoculated (Control)	3.0 a	13.9 a[z]	3.0 a	15.2 a	27.1 a	64.5 a	0.0	0.0
M. phaseolina	3.1 a	12.3 ab	2.7 a	15.2 a	26.9 a	62.1 a	23.6 a	0.0
F. solani	2.2 a	11.8 b	2.7 a	12.9 b	26.8 a	62.0 a	0.0	22.3 a
M. phaseolina + F. solani	2.2 a	11.0 b	2.6 a	12.9 b	26.8 a	56.2 b	22.3 a	27.3 a

[y] Inoculum densities were 20 x 10^3 sclerotia of M. phaseolina/kilogram of soil, and 250 x 10^3 chlamydospores of F. solani/kilogram of soil.

[z] Values (means of 20 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

Table 3. Soybean response and percentage of roots infected 25 days after infesting soil with known quantities of Macrophomina phaseolina and Fusarium solani alone or in combination.[y]

Treatments	Root weight (g)	Shoot weight (g)	Plant height (cm)	Roots with M. phaseolina %	Roots with F. solani %
Non-inoculated (Control)	2.7 a[z]	3.1 a	29.9 a	0.0	0.0
M. phaseolina	2.0 b	2.8 a	26.8 ab	32.6 a	0.0
F. solani	1.6 b	1.8 b	25.0 b	0.0	33.0 a
M. phaseolina + F. solani	0.8 c	1.0 c	21.9 c	26.3 a	43.6 a

[y] Inoculum densities were 40 x 10^3 sclerotia of M. phaseolina/kilogram of soil, and 3,000 x 10^3 chlamydospores of F. solani/kilogram of soil.

[z] Values (means of 20 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

(13%) compared to the non-inoculated control. At low levels of inoculum, plant growth response was not affected by the interaction of M. phaseolina plus R. solani (Table 2), whereas significant reductions from the control in root weight (70%), shoot weight (68%), and plant height (27%) were obtained at high levels of inoculum (Table 3). Root infection by F. solani was greater in soil infested with the combination of M. phaseolina plus F. solani than in soil containing F. solani alone.

After 45 days at low levels of inoculum, root and shoot weights were significantly reduced by F. solani, whereas M. phaseolina only slightly reduced plant growth responses. Macrophomina phaseolina combined with F. solani significantly reduced root weight (21%), shoot weight (15%) and plant height (13%) compared to the non-inoculated control. No significant increase in percentage of root infection by either pathogens occurred when M. phaseolina and F. solani were combined. Since root infection by F. solani and plant stunting were greater in soil infested with M. phaseolina plus F. solani than in soil containing F. solani alone, F. solani interacted additively. Similar results were obtained when these experiments were repeated.

Soybean response to the interaction between Macrophomina phaseolina and Rhizoctonia solani

A significant reduction in root weight, shoot weight, and plant height were obtained with each pathogen alone when compared to the controls except with plant height with M. phaseolina (Table 4). Significant differences in root weight and plant height were obtained between plants exposed to M. phaseolina and R. solani. Macrophomina

Table 4. Soybean response, percentage of roots and hypocotyls infected, disease index, and fungal populations 25 days after infesting soil with known quantities of Macrophomina phaseolina and Rhizoctonia solani alone or in combination.[w]

Treatments	Root weight (g)	Shoot weight (g)	Plant height (cm)	Roots with M. phaseolina (%)	Hypocotyl with R. solani (%)	Disease Index[x] R. solani	Propagules/g of soil M. phaseolina	Propagules/g of soil R. solani
Non-inoculated (Control)	3.5 a[y]	4.2 a	30.2 a	0.0	0.0	0.0	0.0	0.0[z]
M. phaseolina	2.5 b	2.6 b	28.7 a	35.3 a	0.0	0.0	53.0 a	0.0
R. solani	2.0 c	2.6 b	26.1 b	0.0	87.5 a	2.3 a	0.0	0.0
M. phaseolina + R. solani	2.1 c	2.4 c	24.4 c	10.6 b	100.0 a	3.1 a	35.0 b	0.0

[w] Inoculum densities were 40 x 10^3 sclerotia of M. phaseolina/kilogram of soil, and 1,000 sclerotia of R. solani/kilogram of soil.

[x] Disease index rating was based on the hypocotyl decay on a scale of 0 to 4 with 0 = no symptoms and 4 = most severe symptoms (dead plant).

[y] Values (means of 40 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

[z] Plates were heavily infested with Trichoderma spp.

phaseolina infected 35.3% of root segments, caused no hypocotyl decay and caused slight plant stunting; R. solani incited moderate hypocotyl decay, little or no root rot, and moderate plant stunting. The combination of M. phaseolina with R. solani significantly reduced shoot weight and plant height below that for M. phaseolina or R. solani alone.

Compared to the non-inoculated controls, R. solani decreased shoot weight approximately 36% and plant height 14%; whereas R. solani in combination with M. phaseolina reduced shoot weight 42% and plant height 20%.

The percentage of roots infected and the propagules of M. phaseolina per gram of soil were significantly reduced when soil also contained R. solani, but the disease index rating and percentage of hypocotyls infected with R. solani increased when the two fungi were combined. When soil originally infested with R. solani was assayed only colonies of Trichoderma spp. were recovered. Since the severity of the disease caused by R. solani and plant stunting were greater in soil infested with M. phaseolina plus R. solani, M. phaseolina interacted additively with R. solani. Similar results were obtained when these experiments were repeated.

Discussion

Lower inoculum densities of M. phaseolina and R. solani were required for 50% infection of soybean seedlings in autoclaved than in non-autoclaved soil. This was expected because of the apparent lack of competition of other microorganisms with M. phaseolina and R. solani for nutrients in the autoclaved soil.

Lower inoculum densities were required for 50% infection of soybean seedlings by M. phaseolina in this study than have been required for the same level of infection of soybean plants reported by Meyer et al. (77). Approximately 38 x 10^3 sclerotia of M. phaseolina per kilogram of non-autoclaved soil were required for 50% infection of susceptible 'Hood' soybean seedlings grown for 25 days in a growth chamber at 28-35 C with alternate 12 hour periods of fluorescent light (2,000 Lux) and darkness. Meyer et al. (77) found that 50% infection of soybean plants by M. phaseolina required approximately 350 - 370 x 10^3 propagules per kilogram of autoclaved soil mixed with autoclaved silica sand (1:1, v/v) after it had been recolonized by microorganisms for two months. In their experiment 10 'Bragg' soybean seeds were grown in 900 g of infested soil per pot, and all pots were placed for 10 days in growth chamber programmed for 28-30 C and 14-hr day at 1,076 Lux. Several factors, such as variety differences, environmental conditions, isolate differences, amount of infested soil to which plants were exposed, and length of exposure of the plants to the infested soil might have contributed to the discrepancy of the ED50 found in our experiment and that reported by Meyer et al. (77). In our tests, the field soil used had been cropped with soybean for several years and there was also a possibility of synergistic interaction of M. phaseolina with other soil microorganisms contributing to an increase of the seedling infection.

The slope of the log-log transformation of the relationship of number of sclerotia of M. phaseolina to the proportion of infected plants approached 1.0 in non-autoclaved soil, which indicates direct

proportionality; the slope was 1.69 in autoclaved soil. The apparent high slope of M. phaseolina obtained in autoclaved soil might be explained by increased aggressiveness of M. phaseolina resulting from the reduction of antagonistic organisms in the treated soil (34, 86, 87), and by the reduction in competition for nutrients at the onset of sclerotium germination. Weinhold et al. (135) reported that R. solani can effectively utilize exogenous nutrients to increase virulence. This finding provides support for the suggestion that seed exudates may play an important role in attack of seedlings by pathogens. Sclerotia of M. phaseolina have been reported to germinate in the spermosphere of soybean seeds (114), which contains sucrose and fructose (69), compounds known to stimulate germination of sclerotia of M. phaseolina (5). Furthermore, there have been several reports on the reduction of infection of M. phaseolina by antagonistic fungi in several crops (61, 65). Luttrell and Garren (74) also reported that little infection occurred in snap bean in non-treated field soil infested with M. phaseolina but high infection occurred in autoclaved soil. The relatively high slope in non-autoclaved soil alternatively may be due to synergistic interaction between M. phaseolina and other soil microorganisms that recolonized the soil after treatment. There are several reports of interaction of M. phaseolina with plant pathogens increasing disease severity (60, 78, 116).

For R. solani the slopes of the log-log transformations of the relationships between the numbers of sclerotia to the proportions of diseased plants were less than 1.0 in both autoclaved and non-autoclaved soil. Less than direct proportionality of inoculum density to disease

incidence may have occurred because other soil-borne microorganisms residing in non-autoclaved soils or introduced into either of the soils as airborne contaminants acted as antagonists or competitors to the pathogen.

The additive effect of M. phaseolina and F. solani on soybean growth response was influenced greatly by the inoculum densities of the pathogens. When soil with an initial inoculum density of 20×10^3 sclerotia of M. phaseolina/kilogram of soil plus 250×10^3 chlamydospores of F. solani/kilogram of soil was tested, there were no significant reductions in the traits analyzed in comparisons to the pathogens alone. Preliminary experiments with R. solani at 500 sclerotia/kg of soil did not show any interaction of R. solani with M. phaseolina at 20×10^3 sclerotia/kg of soil. However, a significantly higher reduction in root and shoot weight and plant height occurred with corresponding combinations of M. phaseolina with F. solani or R. solani in soil infested at higher inoculum density than in soil infested at the lower inoculum density.

In the additive interaction demonstrated in this study, the number of propagules per gram of soil and the incidence of M. phaseolina on the roots decreased when M. phaseolina was combined with R. solani or F. solani. However, the incidence of R. solani and F. solani tended to increase with the corresponding combination of M. phaseolina with R. solani and F. solani (Tables 3 and 4). Although M. phaseolina seemed to be antagonized by F. solani and R. solani, it still infected soybean roots sufficiently to participate in the reduction of the plant growth response. Schenck and Kinloch (109) presented evidence that

these three pathogens may occur in association, forming a disease complex on soybean roots. Garcia and Mitchell (43) also reported high frequency of isolation of F. solani and R. solani in interactions with M. phaseolina and Pythium myriotylum on peanut pods.

The additive interaction between the corresponding combinations of M. phaseolina with F. solani and R. solani demonstrated in this study may not be operative when environmental conditions are not favorable for M. phaseolina. In preliminary experiments with M. phaseolina low incidences of disease occurred at 20-26 C, severe at temperatures of 28-35 C. Other studies also demonstrated that high temperatures stressed soybeans and reduced plant vigor (3, 19, 38, 77, 100) which favored the speed of tissue colonization by M. phaseolina (86). Our field observations indicated that M. phaseolina was frequently associated with soybeans under 'stress' conditions resulting from the attack of nematodes, insects, poorly fertilized areas, hot weather, and low soil moisture. Thus, with high temperatures and relatively high rate of plant tissue infection, M. phaseolina seemed to assist R. solani and F. solani in the colonization of soybean tissue bringing about a reduction in growth responses. The association of M. phaseolina with other soil-borne, root-infecting fungi in increasing root rot severity has been reported (36, 60, 78, 97, 109, 116).

At the end of the experiment, R. solani could not be recovered from soil, but Trichoderma spp. which were not included in the artificial infestation were recovered. The presence of Trichoderma spp. in soil has been shown to strongly influence the recovery of fungi from roots (42) and soil (46, 57, 136).

Our results also demonstrated that M. phaseolina, F. solani and R. solani alone or in combination could result in considerable reductions of plant growth even when nutritional conditions are not limiting for plant development.

SECTION II

INTERACTIONS AMONG A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS AND SOIL-BORNE ROOT-INFECTING FUNGI ON SOYBEAN

Introduction

Vesicular-arbuscular (VA) mycorrhizal fungi are of great value in promoting phosphorus uptake, plant growth (50, 79, 80) and yield of cultivated crops (7, 103, 107). Furthermore, VA mycorrhiza can influence the severity of disease in several plant-parasite combinations (110). Results obtained previously with interactions involving VA mycorrhiza and root-infecting fungi seem to vary with the species of mycorrhizal fungi involved, races of the plant pathogenic fungi and with plant cultivars.

A few reports indicate that VA mycorrhiza can increase disease severity. Ross (102) in 1972 was the first to report a VA mycorrhizal fungus (Glomus macrocarpus var. geosporus [Nicol. & Gerd.] Gerd. & Trappe) which increased the severity of a plant disease. Eighty-eight percent of the susceptible soybean (Glycine max) plants with mycorrhiza showed internal stem discoloration symptoms due to Phytophthora megasperma var. sojae, race 1, while less than 20% of the nonmycorrhizal plants developed these symptoms. Iqbal et al. (64) in 1977 reported an increase in damping-off of Brassica napus in pots infected with Rhizoctonia solani and endogonaceous spores. In 1978, Davis et al. (28) found that mycorrhizal avocado seedlings were affected more

severely by Phytophthora cinnamomi than were nonmycorrhizal avocado seedlings. Stewart and Pfleger (124) working with poinsettia (Euphorbia pulcherrima 'Annette Hegg Supreme') obtained stunted plants when they were inoculated simultaneously with Glomus mosseae, Pythium ultimum, and Rhizoctonia solani.

Many reports indicate that VA mycorrhizal fungi have no or little effect on disease development. Ramírez (99) in 1974 found that three species of VA mycorrhiza, Glomus macrocarpus var. macrocarpus Tul. & Tul., Gigaspora heterogama (Nicol. & Gerd.) Gerd. & Trappe, and Gigaspora margarita Becker & Hall, had no effect on root infection by Phytophthora palmivora on papaya (Carica papaya). Prior root colonization of Citrus sinensis 'Pineapple' by Glomus fasciculatus did not protect the host against root infection by Phytophthora parasitica (76). Winter (138) noted no differences in the incidence of Gaeumannomyces graminis on the roots of wheat from mycorrhizal and nonmycorrhizal plants. Schönbeck and Dehne (112) working with cotton (Gossypium hirsutum) found the same degree of root infection with Thielaviopsis basicola on mycorrhizal and nonmycorrhizal plants. Alfalfa and citrus plants challenged by Phytophthora megasperma and Phytophthora parasitica, respectively, had no differences in root infection between mycorrhizal (Glomus fasciculatus) and nonmycorrhizal plants (28). Root rot caused by Pythium ultimum was not affected by G. mosseae on soybean (17).

Reports dealing with a mycorrhizal induced decrease in disease severity indicate that there are several mechanisms of host defense involved. The presence of G. mosseae in the roots of onion (Allium

cepa D.) increased the resistance to Pyrenochaeta terrestris (10, 104). Safir (104) found that the percent root infection by P. terrestris was less, and significantly more reducing sugars were present in mycorrhizal than nonmycorrhizal plants. Becker (10) challenged individual mycorrhizal and nonmycorrhizal roots on the same plant with P. terrestris. He found that the inward invasion of the pathogen was restricted more on mycorrhizal roots. Baltruchat and Schönbeck (8) reported a reduction in the population of Thielaviopsis basicola Berk. & Br. by G. mosseae, and a negative correlation between chlamydospore production of T. basicola and mycorrhizal colonization of the roots of tobacco and alfalfa (9). The reduction in chlamydospore production was attributed to free amino-acids in the roots with arginine and citrulline showing the highest increases. Paget (91), working with Cylindrocarpon destructans (Zins) Schol, obtained fewer stunted strawberry plants and reduced root infection by the pathogen when roots were colonized by VA mycorrhiza.

Dehne and Schönbeck (30) studied the influence of the endotrophic mycorrhizal fungus G. mosseae, on the colonization of tomato plants by Fusarium oxysporum Schlect f. sp. lycopersici (Sacc.) Snyd. & Hans. They reported that mycorrhizal tomato plants were damaged less by the pathogen due to a reduced rate of spread of the pathogen. But the mycorrhizal effect was clearly reduced with an increase in inoculum density of the pathogen. Later, Dehne and Schönbeck (31) suggested that Fusarium-wilt resistance in mycorrhizal tomato and cucumber plants was due to a change in cell wall structure. Under the influence of G. mosseae the deposition of lignin in cell walls of the endodermis and the stele was enhanced.

Prior root colonization by VA mycorrhizal fungi in many instances has been shown to have an effect on root disease severity. Chow and Schmitthenner (17) reported that the presence of G. mosseae reduced the number of plants killed by P. megasperma v. sojae 35 days after inoculation. Dehne and Schönbeck (29) indicated that damage to tomato by F. oxysporum f. sp. lycopersici was reduced by prior root colonization by G. mosseae. Prior root colonization by Gigaspora margarita or Glomus macrocarpus has also been reported to reduce the damage caused by P. parasitica to two citrus rootstocks, Carrizo citrange and sour orange (111). Tap root rot was reduced by G. macrocarpus on Carrizo and by G. margarita on sour orange.

This study was undertaken to evaluate the effect of a chlamydosporic species, Glomus mosseae, on the infection of soybean by Macrophomina phaseolina, Fusarium solani, and Rhizoctonia solani.

Materials and Methods

The isolates of M. phaseolina, R. solani, F. solani, and procedures for inoculum production, storage, quantitative estimation of the population from infested soil and evaluation of all experiments were the same as those described in Section I. The G. mosseae isolate used throughout this study was obtained from the Department of Plant Pathology of the University of Florida. The isolate was chosen for its rapid colonization of soybean roots in preliminary tests with other species of VA mycorrhizal fungi.

Chlamydospores of G. mosseae were produced on roots of soybean and bahia grass plants grown in autoclaved field soil in 15-cm

pots in a greenhouse. After 4 months, the chlamydospores were extracted from the soil using the wet-sieving and decanting method described by Gerdemann (47), followed by high speed centrifugation in a 1.3 M sucrose solution for 90 sec. The chlamydospores were then collected on a 240-mesh sieve, back washed into Ringer's solution, and stored in the refrigerator at 5 C.

Numbers of G. mosseae spores in each treatment were estimated by extracting spores as described above from a 25-g subsample of a composite soil sample of three 2.5-cm-diam cores from each of ten replicates. The number of spores in each subsample was counted in 15-cm petri dishes under a dissecting microscope (X40).

Arredondo fine sand collected from a soybean field at the Agronomy Farm, University of Florida, was used throughout this study. After mixing in a cement mixer, part of the soil was stored without any treatment, and part was autoclaved twice for 4 hr at 24 hr intervals at 12 pounds per square inch and 121 C.

Analysis of the soil by the University of Florida Soil Science Department indicated nutrient contents of 91.6 ppm P, 42 ppm K, 450 ppm Ca, 108 ppm Mg, 27 ppm NO_3, 384 ppm Al, 13.6 ppm Fe, 80 ppm Zn, and pH 6.8. Extraction of soil phosphorus and determination of soil pH were accomplished as in Section I.

Root and stem infection was determined by plating twenty, 2.5-cm soybean root pieces, taken at random from each of 10 plants grown in soil infested with M. phaseolina or F. solani, and by plating ten soybean hypocotyls from plants grown in soil infested with R. solani. Roots and hypocotyls were surface sterilized in a 0.5% solution of

sodium hypochlorite before placing on 2% water-agar plates. Each plate of 2% water-agar had three root pieces or one soybean hypocotyl. Twelve days after incubation at 27 C the number of root pieces on which sclerotia of M. phaseolina developed were counted. Soybean hypocotyls from which R. solani grew were counted 36 hr after incubation at 30 C.

The percentage of root colonization by G. mosseae was determined by the method described by Phillips and Hayman (94). A random 2.0 g sample of soybean roots taken from each of 10 plants per treatment was cleared by heating clean root sections in a 50 ml beaker containing hot 10% KOH for 5 min, and then rinsing three to four times with tap water. Each sample was stained in trypan blue for 24-48 hr and stored in water in the refrigerator at 5 C until examined. In each sample the number of root pieces with either vesicles, arbuscles, or surface mycelium of the endomycorrhizal fungus were counted under a dissecting microscope (X40). The percentage of root colonization was obtained by dividing the total number of colonized root sections by the total number of root sections counted.

Seeds of soybean variety 'Hood' were used in this study after they had been surface-sterilized by immersion in 0.5% sodium hypochlorite solution for 2 min, and then rinsed in sterile deionized water. For each treatment, two seeds were planted in each of ten 15-cm clay pots which each contained 2 kg of soil. The plants were maintained in a greenhouse at 28-35 C.

Soil was infested with known numbers of propagules of each pathogen and mixed thoroughly with an electric Hobbart mixer. Water was added

to the soil during the mixing process to give a final water content of 10% (w/w).

The inoculum density of G. mosseae used throughout this investigation was 500 chlamydospores per 15 cm clay pot. This level was shown in preliminary tests to consistently cause moderate amounts of infection. Chlamydospores were distributed in one layer 5.0 cm below the soil surface, and soybeans were seeded 2.5 cm above the mycorrhizal fungus inoculum. The inoculum density of Trichoderma spp. used was 25,000 conidia per gram of soil. Conidia were mixed thoroughly with soil utilizing an electric Hobbart mixer.

The first part of this study was conducted in autoclaved soil. The inoculum densities used were 20 sclerotia of M. phaseolina per gram of soil, 1 sclerotium of R. solani per gram of soil, 3,000 chlamydospores of F. solani per gram of soil. The treatments consisted of each pathogen and G. mosseae alone and G. mosseae in combination with each pathogen. Twenty-five and 45 days after seeding, soybean plants were harvested to determine percentage of root and stem infection by the pathogens, root colonization by G. mosseae, plant height, root and shoot weights, and the population of each organism in the soil.

The second part of this study was conducted in autoclaved and field soil to evaluate the effect of each pathogen alone and in combination with G. mosseae, on soybean seed yield. The inoculum densities of the pathogens were 40 sclerotia of M. phaseolina per gram of field soil; 1 or 2 sclerotia of R. solani per gram of autoclaved and field soil, respectively; and 3,000 chlamydospores of F. solani per gram of autoclaved or field soil. Plants were harvested about 110

days after seeding, and in addition to yield, the percentage of root and stem infection by the pathogens, root colonization by G. mosseae, and the spore population of G. mosseae and other naturally occurring mycorrhizal fungi was also determined.

Results

Soybean Response to the Interaction of Macrophomina phaseolina with Glomus mosseae

Macrophomina phaseolina reduced all plant responses measured, but only root weight at 25 days and shoot weight and plant height at 45 days were significantly lower than the non-inoculated control (Table 5). When G. mosseae was added to soil infested with M. phaseolina, plant response was equivalent to the control. The greatest increase in plant growth response was obtained in soil infested with G. mosseae alone. The correlation coefficient values of the percentage of roots colonized by mycorrhizal and root weight (0.82), shoot weight (0.88), and plant height (0.87) were statistically significant ($P = 0.05$). Significant differences were obtained between date of harvesting for all traits measured. Glomus mosseae did not significantly reduce the percentage of roots infected with M. phaseolina but M. phaseolina in combination with G. mosseae significantly reduced the percentage of root colonization by G. mosseae at 25 and 45 days after planting. The number of sclerotia of M. phaseolina per gram of soil was not affected by the presence of G. mosseae.

In autoclaved soil, G. mosseae alone significantly increased seed weight over the control by 69%, but in combination with M. phaseolina

Table 5. Soybean response, number of sclerotia/g of soil, and percentage of roots infected with Macrophomina phaseolina or colonized with Glomus mosseae alone or in combination 25 and 45 days after infesting soil with known quantities of inoculum.[y]

Treatments	Root weight (g)		Shoot weight (g)		Plant height (cm)		Roots with M. phaseolina (%)		Roots with G. mosseae (%)		Propagules/g of soil of M. phaseolina	
	25	45	25	45	25	45	25	45	25	45	25	45
Non-inoculated (Control)	7.9 a[y]	10.5 bc	4.7 a	13.3 b	24.7 ab	52.0 b	0.0	0.0	0.0	0.0	0.0	0.0
M. phaseolina	6.1 b	9.4 c	3.8 a	7.5 c	21.9 b	36.3 c	36.6 a	50.5 a	0.0	0.0	14.0 a	20.0 a
G. mosseae	8.4 a	12.8 a	4.5 a	14.7 a	25.7 a	57.0 a	0.0	0.0	17.4 a	37.0 a	0.0	0.0
G. mosseae + M. phaseolina	6.1 b	10.8 b	4.3 a	12.9 b	23.6 ab	50.5 b	36.3 a	48.0 a	11.4 b	24.8 b	13.0 a	18.0 a

[y] Inoculum densities were 20 x 10^3 sclerotia of M. phaseolina/kilogram of soil, and 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil).

z Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

G. mosseae increased seed yield only 6% (Table 6). Seed weight was not significantly reduced by M. phaseolina or by Trichoderma spp. The percentage of roots colonized by G. mosseae was significantly reduced by M. phaseolina, but G. mosseae did not influence the percentage of roots infected by M. phaseolina. The presence of M. phaseolina also reduced the chlamydospore numbers of G. mosseae by approximately 60%.

In non-autoclaved soil seed yield was significantly higher with G. mosseae alone than with M. phaseolina (Table 7). Although differences were not significant, seed weight was approximately 27% higher with G. mosseae than in the non-inoculated control plants. Seed yield was reduced by M. phaseolina and Trichoderma spp. approximately 17 and 3%, respectively, in relation to the control.

The percentage of roots colonized by mycorrhizal fungi in non-autoclaved soil formed four distinct statistical groups (Table 7). Macrophomina phaseolina significantly reduced the percentage of roots colonized by G. mosseae and by indigenous mycorrhizal fungi when compared to roots colonized by the indigenous mycorrhizal fungus alone. Trichoderma spp. did not reduce root colonization by indigenous mycorrhizal fungi. Macrophomina phaseolina in combination with G. mosseae fell into the third group and G. mosseae into the last group. The presence of G. mosseae did not reduce significantly the severity of root infection by M. phaseolina. Macrophomina phaseolina reduced the number of chlamydospores of G. mosseae by 26% and also reduced the number of chlamydospores of some indigenous mycorrhiza such as G. etunicatus and G. macrocarpus; it did not affect number of spores formed by

Table 6. Soybean seed yield and percentages of roots colonized by Glomus mosseae or infected with Macrophomina phaseolina after infesting autoclaved soil with known quantities of Trichoderma spp., and Macrophomina phaseolina and Glomus mosseae alone or in combination.[y]

Treatments	Seed weight/ plant (mg)	Seed weight/ plant (% non-inoculated) Loss	Gain	Roots with G. mosseae (%)	Roots with M. phaseolina (%)	Chlamydospores/100 g of soil G. mosseae
Non-inoculated (Control)	310.0 b[z]	--	--	0.0	0.0	0.0
Trichoderma spp.	277.0 b	10.6	00	0.0	0.0	0.0
M. phaseolina	247.0 b	20.4	--	0.0	53.0 a	0.0
G. mosseae	524.0 a	---	69.0	77.6 a	0.0	837.0 a
G. mosseae + M. phaseolina	329.0 b	---	6.2	49.4 b	56.0 a	334.0 a

[y] Inoculum densities were 40 x 10^3 sclerotia of M. phaseolina/kilogram of soil; 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil); 25,000 x 10^3 conidia of Trichoderma spp./ kilogram of soil.

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

Table 7. Soybean seed yield, percentage of roots colonized by mycorrhizae and infected with Macrophomina phaseolina, and populations of mycorrhizal fungi 110 days after infesting non-autoclaved soil with known quantities of Trichoderma spp. and of Macrophomina phaseolina and Glomus mosseae alone or in combination.[y]

Treatments	Seed weight/ plant (mg)	Seed weight/ plant (% non-inoculated)		Roots with mycorrhizae (%)	Roots with M. phaseolina (%)	Spores of mycorrhizal fungi/100 g of soil			
		Loss	Gain			Glomus mosseae	Glomus etuni-catus	Glomus macro-carpus	Gigaspora margarita
Non-inoculated (control)	225.0 ab[z]	---	---	49.1 c	0.0	0.0	311.0 a	18.0 a	38.0 a
Trichoderma spp.	219.0 ab	2.7	---	46.4 c	0.0	0.0	467.0 a	6.0 a	34.0 a
M. phaseolina	188.0 b	16.5	---	30.1 d	84.5 a	0.0	179.0 a	0.0	41.0 a
G. mosseae	285.0 a	---	26.7	78.8 a	0.0	87.0 a	891.0 a	0.0	41.0 a
G. mosseae + M. phaseolina	242.0 ab	---	7.5	57.0 b	77.0 a	64.0 a	573.0 a	0.0	29.0 a

[y] Inoculum densities were 150 x 10^3 sclerotia of M. phaseolina/kilogram of soil, 500 chlamydospores of G. mosseae/15 cm clay pot; (2,000 g of soil), and 25,000 x 10^3 conidia of Trichoderma spp./ kilogram of soil.

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

Gigaspora margarita. Similar results were obtained when the experiment was repeated.

Soybean Response to the Interaction of Rhizoctonia solani with Glomus mosseae

Rhizoctonia solani reduced all plant responses measured, but only root weight and plant height at 25 and 45 days were significantly lower than the non-inoculated control (Table 8). When G. mosseae was added to soil infested with R. solani, plant response was equivalent to the control. The greatest increase in plant growth response was obtained with soil infested with G. mosseae alone. The correlation coefficient values of the percentage of roots colonized by mycorrhiza and root weight (0.87), shoot weight (0.84), and plant height (0.92) were statistically significant ($P = 0.05$). Significant differences ($P = 0.05$) were obtained between date of harvesting for all traits analyzed. Glomus mosseae did not significantly reduce the percentage of hypocotyl infection by R. solani or the disease index, but R. solani significantly reduced the percentage of root colonization by G. mosseae at 25 or 45 days after planting.

In autoclaved soils, seed weights fell into four distinct groups (Table 9). Glomus mosseae alone significantly increased seed weight over the control (66%), but plants grown in soil with R. solani and G. mosseae only had 39% greater seed weight than the controls. Seed yield was significantly reduced (30%) by R. solani alone as compared to the non-inoculated controls. The percentage of roots colonized by G. mosseae was significantly lower in the presence of R. solani, but G. mosseae did not influence the disease index rating. In

Table 8. Soybean response, percentage of hypocotyls infected with Rhizoctonia solani, disease index, and percentage of roots colonized with Glomus mosseae alone or in combination with R. solani 25 and 45 days after infesting soil with known quantities of inoculum.[x]

Treatments	Root weight (g)		Shoot weight (g)		Plant height (cm)		Hypocotyl with R. solani (%)		Disease Index[y] R. solani		Roots with G. mosseae (%)	
	25	45	25	45	25	45	25	45	25	45	25	45
Non-inoculated (Control)	3.5 b[z]	7.1 a	3.9 b	5.9 b	27.2 b	44.0 c	0.0	0.0	0.0	0.0	0.0	0.0
R. solani	2.6 c	5.9 b	3.5 b	5.7 b	24.4 c	35.5 d	53.9 a	90.0 a	2.3 a	2.8 a	0.0	0.0
G. mosseae	4.7 a	7.1 a	4.7 a	7.7 a	31.0 a	55.7 a	0.0	0.0	0.0	0.0	12.2 a	33.0 a
G. mosseae + R. solani	3.0 b	7.0 a	4.6 a	6.5 b	28.6 ab	48.4 b	42.0 a	80.0 a	2.0 a	2.4 a	9.2 b	30.6 b

[x] Inoculum densities were 1,000 sclerotia of R. solani/kilogram of soil, and 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil).

[y] Disease index rating was based on a scale of 0 to 4 with 0 = no symptoms and 4 = most severe symptoms (dead plant).

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

Table 9. Soybean seed yield, percentage of roots colonized by Glomus mosseae, disease index of Rhizoctonia solani and populations of Glomus mosseae 110 days after infesting autoclaved soil with known quantities of Rhizoctonia solani and Glomus mosseae alone or in combination.[x]

Treatments	Seed weight/ plant (mg)	Seed weight/ plant (% non-inoculated) Loss	Gain	Roots with G. mosseae (%)	Disease Index[y] R. solani	Chlamydospores/100 g of soil G. mosseae
Non-inoculated (control)	283.0 c[z]	---	---	0.0	0.0	0.0
R. solani	197.0 d	30.4	---	0.0	2.7 a	0.0
G. mosseae	469.0 a	---	65.7	74.2 a	0.0	198.0 a
G. mosseae + R. solani	392.0 b	---	38.5	65.6 b	2.6 a	191.0 a

[x] Inoculum densities were 1,000 sclerotia of R. solani/kilogram of soil, and 500 chlamydospores of G. of G. mosseae/15 cm of clay pot (2,000 g of soil).

[y] Disease index rating was based on a scale of 0 to 4 with 0 = no symptoms and 4 = most severe symptoms (dead plants).

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

non-autoclaved soil, R. solani reduced seed yield 10%, and G. mosseae increased seed yield 5% in relation to the control, but the differences were not statistically significant (Table 10).

Rhizoctonia solani did not influence the percentage of roots colonized by G. mosseae but increased the percentage for indigenous mycorrhiza when compared to roots colonized by these mycorrhiza alone (Table 10). The presence of G. mosseae did not reduce significantly the disease index rating by R. solani. Rhizoctonia solani did not reduce the number of chlamydospores of G. mosseae and spores of Gigaspora margarita, but it did reduce the number of chlamydospores of Glomus etunicatus by 40%. Similar results were obtained when the experiment was repeated.

Soybean Response to the Interaction of Fusarium solani with Glomus mosseae

Fusarium solani reduced all plant responses measured, but only shoot weight, root weight and plant height after 45 days were significantly lower with F. solani than in the non-inoculated control (Table 11). When G. mosseae was added to soil infested with F. solani, plant response was equivalent to the control. The greatest increase in plant response was obtained with soil infested with G. mosseae alone. The correlation coefficient values of the percentage of roots colonized by mycorrhiza and root weight (0.78), shoot weight (0.76) and plant height (0.73) were statistically significant ($P = 0.05$). Significant differences were obtained between harvest dates. Glomus mosseae did not influence either the percentage of roots infected or the number of propagules per gram of soil of F. solani, but F. solani greatly reduced the percentage of roots colonized by G. mosseae.

Table 10. Soybean seed yield, percentage of roots colonized by mycorrhizae, disease index of Rhizoctonia solani, and population of mycorrhizal fungi 110 days after infesting non-autoclaved soil with known quantities of Rhizoctonia solani and Glomus mosseae alone or in combination.[x]

Treatments	Seed weight/ plant (mg)	Seed weight/ plant (% non-inoculated) Loss	Gain	Roots with mycorrhizae (%)	Disease Index[y] R. solani	Spores of mycorrhizal fungi/ 100 g of soil Glomus mosseae	Glomus etunicatus	Gigaspora margarita
Non-inoculated (control)	307.0 a[z]	---	---	44.2 c	1.2 a	0.0	586.0 a	3.0 a
R. solani	277.0 a	9.7	---	55.5 b	2.1 a	0.0	1197.0 a	4.0 a
G. mosseae	323.0 a	---	5.2	78.1 a	1.3 a	103.0 a	662.0 a	11.0 a
G. mosseae + R. solani	317.0 a	---	3.2	75.8 a	2.0 a	175.0 a	349.0 a	31.0 a

[x] Inoculum densities were 2,000 sclerotia of R. solani/kilogram of soil, and 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil).

[y] Disease index rating was based on a scale of 0 to 4 with 0 = no symptoms and 4 = most severe symptoms (dead plant).

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

Table 11. Soybean response, percentages of roots colonized by Glomus mosseae and infected with Fusarium solani, and populations of Fusarium solani 25 and 45 days after infesting soil with known quantities of Fusarium solani and Glomus mosseae alone or in combination.

Treatments	Root weight (g)		Shoot weight (g)		Plant height (cm)		Roots with G. mosseae (%)		Roots with F. solani (%)		Propagules/g of soil of F. solani
	25	45	25	45	25	45	25	45	25	45	45
Non-inoculated (control)	2.4 ab[y]	6.1 c	3.8 b	7.9 b	30.5 ab	40.3 b	0.0	0.0	0.0	0.0	0.0
F. solani	1.6 b	3.7 d	3.0 b	4.0 c	28.8 b	35.4 c	0.0	0.0	34.0 a	55.0 a	1600.0 a
G. mosseae	4.0 a	13.9 a	5.5 a	13.0 a	32.0 a	46.4 a	19.0 a	57.0 a	0.0	0.0	0.0
G. mosseae + F. solani	2.3 ab	9.3 b	4.0 ab	8.1 b	31.8 a	44.4 a	15.0 a	44.0 b	30.0 a	52.0 a	1500.0 a

[x] Inoculum densities were 3,000 x 10^3 chlamydospores of F. solani/kg of soil, and 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil)

[y] Values (means of 10 plants) in vertical column followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

In autoclaved soil, G. mosseae alone or in combination with F. solani increased seed weight 12 to 14% over the non-inoculated control, but the differences were not statistically significant (Table 12). Fusarium solani alone reduced seed weight by 19% but the difference was not statistically significant. The percentage of roots colonized by G. mosseae was significantly reduced by F. solani, but G. mosseae did not influence the percentage of roots infected by F. solani significantly. Fusarium solani reduced the number of chlamydospores of G. mosseae by 14%.

In non-autoclaved soil, G. mosseae alone increased seed weight over the control by 17%, but plants grown in soil containing both F. solani and G. mosseae had 13% greater seed weight than that of the control plants (Table 13). Seed yield was reduced by F. solani by 15%. The percentage of roots colonized by mycorrhizal fungi was reduced significantly by F. solani, but G. mosseae did not influence the percentage of roots infected by Fusarium spp. The number of chlamydospores of G. mosseae and spores of Gigaspora margarita was reduced by Fusarium solani, but the number of chlamydospores of Glomus etunicatus increased in a presence of F. solani.

Significant correlations were obtained between seed yield and plant growth response at 45 days for almost all traits analyzed either in autoclaved or non-autoclaved soil (Table 14). At 25 days, correlations between seed yield and plant growth approached significance. These results suggest that the evaluation of soybean growth response based mainly on root weight and plant height a few days before the blooming stage can predict the performance of the treatments in relation

Table 12. Soybean seed yield, percentages of roots colonized by Glomus mosseae and infected with Fusarium solani, and populations of Glomus mosseae 110 days after infesting autoclaved soil with known quantities of Fusarium solani and Glomus mosseae alone or in combination.[x]

Treatments	Seed weight/ plant (mg)	Seed weight/ plant (% non-inoculated) Loss	Gain	Roots with G. mosseae (%)	Roots with F. solani (%)	Chlamydospores/ 100 g of soil G. mosseae
Non-inoculated (control)	283.0 ab[y]	---	---	0.0	0.0	0.0
F. solani	228.0 b	19.4	---	0.0	60.0 a	0.0
G. mosseae	318.0 a	---	12.4	75.1 a	0.0	572.0 a
G. mosseae + F. solani	322.0 a	---	13.7	56.8 b	54.5 a	492.0 a

[x] Inoculum densities were 3,000 x 10^3 chlamydospores of F. solani/kilogram of soil, and 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil).

[y] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

Table 13. Soybean seed yield, percentages of roots colonized by mycorrhizae and infected with Fusarium[x] spp., and populations of mycorrhizal fungi 110 days after infesting non-autoclaved soil with known quantities of Fusarium solani and Glomus mosseae alone or in combination.[y]

Treatments	Seed weight/ plant	Seed weight/ plant (% non-inoculated)		Roots with mycorrhizae	Roots with Fusarium spp	Spores of mycorrhizal fungi/100 g of soil			
	(mg)	Loss	Gain	(%)	(%)	Glomus mosseae	Glomus etunicatus	Glomus clarus	Gigaspora margarita
Non-inoculated	296.0 ab[z]	--	--	61.1 b	10.0 b	0.0	241.0 a	1.0 a	65.0 a
F. solani	252.0 b	14.8	--	58.4 b	65.8 a	2.0 a	1412.0 a	11.0 a	32.0 a
G. mosseae	345.0 a	--	16.5	78.6 a	12.6 b	104.0 a	471.0 a	4.0 a	66.0 a
G. mosseae + F. solani	335.0 a	--	13.1	64.2 b	63.8 a	60.0 a	482.0 a	5.0 a	15.0 a

[x] Fusarium species recovered from roots included F. solani and F. oxysporum.

[y] Inoculum densities were 3,000 x 10^3 chlamydospores of F. solani/kilogram of soil, and 500 chlamydospores of G. mosseae/15 cm of clay pot (2,000 g of soil).

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

Table 14. Correlation coefficient values between plant growth responses at 45 days after planting and soybean seed yields of plants grown in autoclaved or non-autoclaved soil containing *Macrophomina phaseolina*, *Rhizoctonia solani* or *Fusarium solani*.

Parameters Analyzed	Soybean Seed Yield					
	Macrophomina phaseolina		*Rhizoctonia solani*		*Fusarium solani*	
	Autoclaved soil	Non-autoclaved soil	Autoclaved soil	Non-autoclaved soil	Autoclaved soil	Non-autoclaved soil
Root weight (g)	.99*	.98*	.76*	.98*	.85*	.97*
Shoot weight (g)	.76	.89*	.90*	.72	.82	.88*
Plant height (cm)	.91*	.94*	.95*	.95*	.95*	.99*

* Significant at (P = 0.05).

to seed yield. Similar results were obtained when the experiment was repeated.

Discussion

Our results demonstrated that the three pathogens differed in their major effects on plant response. Macrophomina phaseolina reduced shoot weight and plant height most, R. solani reduced root weight and plant height most, while F. solani reduced all three plant responses. Rhizoctonia solani seemed to be more aggressive than M. phaseolina and F. solani since a significant reduction in root weight and plant height occurred within 25 days after planting. For M. phaseolina and F. solani, the most striking reduction in plant growth response occurred at 45 days. These findings agree with reports of damage by these fungi either under greenhouse condition or in the field (16, 105, 106, 118).

Although M. phaseolina can infect soybean plants at any age (41, 63) the disease is more pronounced at the end of the growing season (117, 139). Our results showed that once soybean plants become infected with M. phaseolina at 20 x 10^3 sclerotia per kilogram of soil early in the season, there was a gradual decline in plant vigor with age. No symptom other than plant stunting was observed above ground. The soybean decline and the increase in disease severity were followed by a substantial increase in the population of M. phaseolina. These results agree with those reported in the literature in which the severity of root rot of soybean was related directly to the population of germinable sclerotia of M. phaseolina in soil, and soybean yields were inversely related to the severity of the disease (14, 77, 115, 134).

This study showed that R. solani at 1,000 sclerotia/kg of soil can cause a reduction in plant growth at the seedling stage, which was reflected mainly in the reduction of root weight and plant height. Orellana et al. (89) also observed a reduction of root and top weights of soybean at high inoculum densities of R. solani. The slightly higher incidence of disease caused by R. solani at 25 as compared to 45 days after planting suggests that plants became resistant as they get older. Luttrell and Garren (74) suggested that disease of bean plants by R. solani does not occur after the plants have passed the blooming stage. Christou (18) also reported that the increasing resistance of bean plants with age prevented additional infection by R. solani and could check further parasitic activity in the already invaded tissues.

Several surveys indicated that F. solani occurs on field soybeans in the roots or stems of susceptible plants (37, 88). Pathogenicity of F. solani on soybean was demonstrated by Cheng (16) in 1977 by a stem injection inoculation method using 8.2×10^4 spores/ml. The pathogen was reported to cause root rot of the tap and lateral roots and vascular discoloration at 30 days after inoculation. Our studies showed that F. solani did not cause discoloration of the vascular system but rather produced small brown spots on the root system, and occasionally rot occurred at the base of the stem. The level of inoculum and the method of inoculation may have accounted for the differences between the two studies. The studies by both Cheng (16) and the present investigation, however, indicate that root and shoot growth of soybean are reduced by F. solani.

When M. phaseolina, R. solani, or F. solani were combined with G. mosseae, there was an increase in the soybean growth response in relation to the individual pathogens alone. Mycorrhizal plants could withstand the stress of infection by each pathogen better than non-mycorrhizal plants, since the severity of root and hypocotyl infection by M. phaseolina, R. solani, and F. solani was not significantly reduced by G. mosseae. These results possibly occurred because of increased nutrient uptake by mycorrhizal plants. Davis et al. (27) found that concentrations of phosphorus were lower in plants infected with V. dahliae alone than in plants infected with both V. dahliae Kleb. and G. fasciculatus (Thax.) Gerd. and Trappe. Lambert et al. (73) also reported that the concentrations of phosphorus, potassium, zinc, copper and iron were higher in mycorrhizal than in non-mycorrhizal treatments. It is also known that root colonization by mycorrhizal fungi can greatly influence disease caused by root infecting fungi (28, 64, 102, 124), and usually mycorrhizal plants yielded more and were less affected by pathogenic fungi than non-mycorrhizal plants (8, 9, 10, 17, 28, 29, 30, 31, 64, 91, 104, 111, 112, 138).

The presence of the pathogens and G. mosseae together reduced the effectiveness of the mycorrhizal fungus and consequently reduced yield from that obtained with G. mosseae alone. In fact, there were considerable reductions in the percentages of roots colonized by G. mosseae in combination with M. phaseolina, R. solani, or F. solani compared with G. mosseae alone. This is the first report of M. phaseolina and R. solani causing a significant reduction in root

colonization by G. mosseae. Only a few reports indicate the effect of soil borne pathogenic fungi on the development of VA-mycorrhizal fungi (27, 76).

In autoclaved soil, reductions in seed yield of 20, 30, and 19% were obtained with M. phaseolina, R. solani, and F. solani, respectively, compared to the non-inoculated control plants. But when G. mosseae was introduced with these pathogens there was an increase in seed weight of about 6, 13, and 38%, respectively, over that in the controls. In non-autoclaved soil there was less damage by these pathogens than in autoclaved soil, and seed yield reductions caused by the pathogens were offset by the presence of G. mosseae. Thus, the damage caused by the individual pathogens seemed to be compensated for by the introduction of G. mosseae either in autoclaved or non-autoclaved soil. Since the increase in seed yield in the presence of M. phaseolina, R. solani, or F. solani plus G. mosseae cannot be explained by the effect of G. mosseae on pathogen infection or disease severity, it is suggested that the mycorrhizal plants with their increased nutrients (50, 76, 79) were better able to withstand the attack of the pathogens than were nonmycorrhizal plants. The effectiveness of the introduced mycorrhizal fungi in field soil apparently can be greatly reduced by competition with the well established indigenous mycorrhiza and pathogenic organisms for infection sites on the host plant.

In autoclaved soil seed yield was significantly greater ($P<0.05$) in plants exposed to G. mosseae than in non-inoculated plants in two of three experiments. However, in non-autoclaved soil differences in

seed yield in soils with and without G. mosseae added were not significant and were lower than those in autoclaved soil. Several other reports also showed that the effect of VA-mycorrhizal fungi on yields were less in non-autoclaved than in autoclaved soil (48, 82, 83). Furthermore, there have been no reports of significant increases in seed yield in field soils artificially infested with VA-mycorrhizal fungi as compared to noninoculated controls (7, 11, 66, 70).

The variation in seed yields among experiments in the same soil treatment may have been due to differences in temperature, daylength, and light intensity, since the experiments were performed at different times of the year. These environmental factors are known to affect the ability of soybeans to synthesize photosynthetic products and consequently affect yield (45, 122, 127). The fact that photosynthetically labelled carbon travels from the host to the mycorrhizal spores (59) and to the mycelium (21) where it was detected in lipids, indicates that a reduced supply of photosynthate may have interfered in the uptake of nutrients by the mycorrhizal fungi. Hayman (55), using onions, reported that high light intensity increased the plant growth, carbohydrate content, and the mycorrhizal effect; but under poor light and lower temperature plant growth was not stimulated. The largest growth enhancement of onion plants by VA-mycorrhizal fungi was obtained in conditions of light and temperature optimal for the growth of onions.

Although there were high populations of some indigenous mycorrhizal fungi in non-autoclaved soil at harvest, seed weight in soil not infested with G. mosseae was lower than in soil artificially infested with G. mosseae. These findings suggest that either soybean roots were

slowly or poorly colonized by indigenous mycorrhizal fungi in the early growth stages, or that the indigenous species were not effective in promoting plant growth responses, or both. The spore populations of indigenous mycorrhizal fungi (Gigaspora margarita, Glomus etunicatus, Glomus clarus and Glomus macrocarpus) were so low at the beginning of the experiment that no spores could be detected by the wet seivings technique. Mosse (81) reported that inoculum density in the soil, rather than its phosphate status, seemed to determine responses to VA-mycorrhizalinoculation. The poor performance of indigenous VA mycorrhizal fungi was indicated in a preliminary experiment in which Florida isolates of G. margarita, G. macrocarpus, and G. etunicatus were not as effective as G. mosseae in promoting soybean plant growth responses. Powell (95), however observed that in 11 soils from well developed pastures, the indigenous mycorrhizal fungi were more efficient in increasing white clover growth than an introduced mycorrhizal fungus.

Results from this study showed that mycorrhizal fungi can be beneficial to plant growth even with high levels of phosphorus. Similar results have been reported with levels of phosphorus higher than used in this study. Powell (95) reported that although the response to mycorrhizal colonization decreased as soil fertility increased, indigenous mycorrhizal fungi were still beneficial in most forest soils even with 120 ppm of P. Yost and Fox (141) indicated that the indigenous mycorrhizal fungi were effective in promoting P uptake by soybean and several legumes until the soil solution reached 86 ppm of P or higher compared with the plant uptake in absence of indigenous mycorrhizal fungi.

SECTION III

EFFECT OF ROOT ROT PATHOGENS AND THE VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS GLOMUS MOSSEAE ON THE ESTABLISHMENT OF RHIZOBIUM JAPONICUM ON SOYBEAN

Introduction

Upon infection of the appropriate legume, Rhizobium species cause the formation of root nodules and can participate in the symbiotic fixation of N_2 (132). Commonly, 25 to 60% of the total nitrogen needed by the soybean plant is supplied by symbiotic nitrogen fixation (54). Sinclair and Dewit (119), in an analysis of 24 legumes for N_2 requirement, found that soybeans had the highest nitrogen requirement for seed production. Indeed, there are several studies that show a positive correlation between symbiotic N_2 fixation and yield, particularly when efficient R. japonicum strains are used (121, 132).

Only in the last decade have researchers initiated studies between Rhizobium japonicum and root infecting fungi or VA mycorrhizal fungi. Results are contradictory in the few studies dealing with the interaction of Rhizobium spp. with rhizosphere organisms. Chou and Schmitthenner (17) found that soybean plants infected by Pythium ultimum and Phytophthora megasperma var sojae race 1 developed normal bacterial root nodules. On the other hand, Orellana et al. (89) demonstrated that R. solani significantly reduced top and nodule

weights of two soybean varieties. Later, Orellana and Worley (90) showed that the cell disfunction in young nodules of R. japonicum on soybean grown in the presence of R. solani may have been caused by toxic fungal metabolites that diffuse throughout the nodule. Rhizobium spp. have also been reported to be antagonistic toward root pathogens. In a greenhouse experiment Phytophthora root rot of soybean was lessened when Rhizobium japonicum was applied to the potted soil immediately after planting (128). "In vitro" studies showed that the bacteria were constantly present within the fungal hyphae of P. megasperma, which suggested that Rhizobium species living saprophytically in soil may reduce Phytophthora root rot by parasitizing hyphae of the fungus.

Research in the last few decades with VA mycorrhizal fungi has established that these symbiotic organisms can improve plant growth through increased uptake of phosphorus, especially in soils of low fertility (50, 79, 80). Ross (101) in 1971 suggested that nonmycorrhizal soybean roots were inefficient phosphate-absorbing organs and that mycorrhizal infection promoted the uptake of phosphate. Legumes growing in uncultivated or agricultural soils normally form VA mycorrhiza (4, 67). Asai (4) in 1944 demonstrated that several legumes grew poorly and failed to nodulate in autoclaved soil unless they were mycorrhizal. Since the publication of Asai's results, the relationships between VA mycorrhizae and enhanced phosphorus uptake or improved host growth have been well established for many leguminous plants (23, 24, 26, 79, 80, 84) including soybeans (65, 101, 103). Asai (4) in 1944 found that only four of 59 legume species examined

were not mycorrhizal, and Strzemska (125) in 1975 confirmed the widespread occurrence of mycorrhiza in 20 species in the Papilionaceae.

The addition of VA-mycorrhizal fungi to fumigated soil has increased soybean yields (103, 107). The yield of nodulated soybeans grown in fumigated soil inoculated with Glomus macrocarpus var. geospora was increased 29% over the non-inoculated controls (103). Schenck and Hinson (108) in 1973, working in a methyl bromide-fumigated soil in the field found that a mycorrhizal fungus significantly increased seed yield, seed protein, and leaf nitrogen of the nodulating, but not the non-nodulating soybean cultivar used.

Recently the interaction between Glomus fasciculatus and Rhizobium japonicum was studied in the field by Bagyaraj et al. (7). They found that shoot dry weight and shoot nitrogen content were increased in soil infested with both symbionts as compared to treatments with either G. fasciculatus or R. japonicum alone; this suggests that VA mycorrhizae stimulate nodulation and nitrogen fixation in field grown soybean. Most of the studies on the interaction between Rhizobium species and VA mycorrhizal fungi do not consider root infecting fungi which could alter the beneficial effects of the symbiotic organisms. This study was undertaken to determine the effect of root infecting fungi Macrophomina phaseolina, Rhizoctonia solani, and Fusarium solani on the interaction of G. mosseae on R. japonicum on soybean development.

Materials and Methods

The isolates of the three pathogens, procedures for production and storage of inoculum, quantitative estimation of their population

in infested soil, and methods for evaluation of the percentages of infection by the pathogens were the same as those described in Section I. The isolate of G. mosseae used in this study, the procedures for chlamydospore production and storage, evaluation of root colonization and the method of inoculation were the same as those described in Section II. The autoclaved soil used throughout this study was the same as that used in experiments described in Section II.

The combination of organisms and their inoculum densities per gram of soil were G. mosseae at 500 chlamydospores per 15-cm pot plus M. phaseolina at 40 sclerotia plus Rhizobium japonicum at 5 mg/g of seed; G. mosseae plus R. solani at 1 sclerotium plus R. japonicum; G. mosseae plus F. solani at 3,000 chlamydospores plus R. japonicum; M. phaseolina plus R. japonicum; R. solani plus M. japonicum; F. solani plus R. japonicum, and G. mosseae plus R. japonicum. All combinations of the above organisms were compared on both the nodulating cultivar Hardee and a non-nodulating isoline of Hardee which differed from Hardee in only one gene which conferred resistance to infection by Rhizobium japonicum.

Seed of nodulating and non-nodulating Hardee cultivars were surface sterilized by immersion in 0.5% sodium hypochlorite solution for 2 min, and rinsed in sterile deionized water. They were then treated before planting with commercial R. japonicum inoculum.

All experiments were conducted with two soybean plants in 2 kg of autoclaved soil in each of ten 15-cm clay pots per treatment. The plants were maintained in a greenhouse at 28-35 C. Soil was infested with each pathogen and mixed thoroughly with an electric Hobbart mixer.

Water was added to the soil during the mixing process to give a final water content of 10% (wt/wt). Forty-five days after seeding, soybean plants were harvested to determine the percentage of root and stem infection, plant height, root and shoot weights, and number and weight of R. japonicum nodules; the shoots were analyzed for levels of N, P, K, Ca, Mg, Zn, Mn, Fe and Cu.

Results

Interaction Among Macrophomina phaseolina, Glomus mosseae and Rhizobium japonicum

There were no significant differences between the means of nodulating and non-nodulating plants of the cultivar Hardee in all parameters studied (Table 15). Inoculation with M. phaseolina plus R. japonicum resulted in significantly less root weight than other treatments for both Hardee and its non-nodulating isoline. Exposure to M. phaseolina plus R. japonicum decreased the mean root weight of nodulating and non-nodulating plants 35% compared with R. japonicum treatment alone, but shoot weight and plant height did not differ significantly from plants exposured to R. japonicum alone. However, plants treated with M. phaseolina plus R. japonicum did differ significantly from those treatments where G. mosseae was present in almost all parameters analyzed. Plants exposed to G. mosseae plus R. japonicum showed the highest root and shoot weights, and plant heights.

Fewer roots were infected by M. phaseolina when G. mosseae was present, and more roots for both nodulated and non-nodulated plants were colonized by G. mosseae in the absence of M. phaseolina, but these differences were not statistically significant.

Table 15. Soybean response, nodule number and weight, and percentage of roots infected with Macrophomina phaseolina and colonized by Glomus mosseae in combination with Rhizobium japonicum on nodulating and non-nodulating Hardee[u] soybeans 45 days after infesting soil with known quantities of inoculum.[y]

Treatments	Root Weight (g)	Shoot Weight (g)	Plant Height (cm)	Roots with M. phaseolina (%)	Roots with G. mosseae	Nodules/plant Number	Nodules/plant Weight (mg)
			Nodulated				
R. japonicum	7.3 b[z]	11.6 b	49.8 cd	0.0	0.0	28.0 a	204.0 b
M. phaseolina + R. japonicum	4.2 c	8.4 c	49.5 cd	56.0 a	0.0	17.0 b	139.0 b
G. mosseae + R. japonicum	12.2 a	16.8 a	61.7 a	0.0	52.7 a	34.0 a	443.0 a
M. phaseolina + G. mosseae + R. japonicum	7.2 b	15.6 a	57.0 ab	47.0 a	46.9 a	26.6 ab	118.0 b
			Non-nodulated				
R. japonicum	7.8 b	8.7 c	45.0 d	0.0	0.0	0.0	0.0
M. phaseolina + R. japonicum	5.6 c	6.3 c	47.2 d	48.0 a	0.0	0.0	0.0
G. mosseae + R. japonicum	13.0 a	16.9 a	57.0 a	0.0	56.0 a	0.0	0.0
M. phaseolina + G. mosseae + R. japonicum	12.6 a	12.8 b	53.2 bc	46.0 a	47.0 a	0.0	0.0

[y] Inoculum densities were 20 x 10^3 sclerotia of M. phaseolina/kilogram of soil, 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil), and 0.5% of R. japonicum (wt/wt) of the seeds.

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

Macrophomina phaseolina in combination with R. japonicum reduced significantly the number and weight of nodules in relation to the G. mosseae plus R. japonicum treatment. The combination of M. phaseolina either with R. japonicum or with the bacterium plus G. mosseae always reduced the number and weight of nodules in relation to R. japonicum alone. Macrophomina phaseolina in combination with R. japonicum reduced the number (39%) and weight (32%) of nodules per plant in relation to R. japonicum alone.

The concentrations of elements in nodulated and non-nodulated plant shoots did not differ significantly, but they differed in the total amount of Ca, Mg, K and N present in the shoots (Table 18). The concentration of the elements in the shoots was neither reduced by M. phaseolina nor increased significantly by G. mosseae (Table 19). However, the total amount of elements present in the shoots was considerably reduced by M. phaseolina and greatly increased by G. mosseae. Significant increases in the amount of N, P, K, Ca, Mg, and Cu present in the shoots for both nodulated and non-nodulated plants were obtained with G. mosseae with or without M. phaseolina (Table 20).

Interaction Among Rhizoctonia solani, Glomus mosseae and Rhizobium japonicum

There were no significant differences among non-nodulating and nodulating plants of the cultivar Hardee in all of the parameters studied except for plant shoot weight (Table 16).

Inoculation with R. solani plus R. japonicum resulted in significantly lower root weight, and inoculation with G. mosseae plus R. japonicum resulted in significantly higher root weight than the other

treatments. The mean root weight of nodulated and non-nodulated plants was decreased 31% by inoculation with R. solani plus R. japonicum compared to R. japonicum alone. For the nodulated plants, the highest and the lowest shoot weights were obtained with G. mosseae plus R. japonicum and R. solani plus R. japonicum, respectively. Plant height of the nodulated plants inoculated with R. solani plus R. japonicum was not significantly different from those plants inoculated with R. japonicum alone, but it was significantly less than in plants inoculated with G. mosseae plus R. japonicum with or without R. solani.

In the presence of G. mosseae there were fewer soybean hypocotyls infected by R. solani and a lower disease index rating than in the absence of G. mosseae, but these differences were not statistically significant. Rhizoctonia solani decreased the percentage of roots colonized by G. mosseae in both nodulated and non-nodulated plants, but only with the non-nodulated plants were these differences statistically significant.

The number and weight of nodules per plant for the R. solani plus R. japonicum treatment showed an inverse relationship to G. mosseae plus R. japonicum treatment. Rhizoctonia solani in combination with R. japonicum reduced nodule numbers 35% and nodule weight 68% compared with R. japonicum treatment alone. Rhizoctonia solani in combination with R. japonicum significantly reduced the number and weight of nodules per plant compared with treatments including G. mosseae, even though differences were not significant as compared to the R. japonicum alone.

The disease index rating of R. solani on hypocotyls was correlated negatively with the mean weight of nodules (-0.46; $P = 0.05$), and

Table 16. Soybean response, nodule number and weight, disease index, percentage of hypocotyls infected with R. solani, and percent of roots colonized by G. mosseae in combination with Rhizobium japonicum 45 days after inoculation with known quantities of inoculum.[x]

Treatments	Root Weight (g)	Shoot Weight (g)	Plant Height (cm)	Hypocotyl with R. solani (%)	Disease Index[y]	Roots with G. mosseae (%)	Nodules/plant	
							Number	Weight (mg)
				Nodulated				
R. japonicum	5.4 de	11.5 b[z]	58.1 ab	0.0	0.0	0.0	40.0 bc	165.0 c
R. solani + R. japonicum	3.5 f	9.8 b	51.7 b	100.0 a	3.0 a	0.0	26.5 c	52.0 c
G. mosseae + R. japonicum	8.7 a	17.1 a	63.0 a	0.0	0.0	54.9 a	69.4 a	839.0 a
R. solani + G. mosseae + R. japonicum	7.3 bc	11.6 b	61.0 a	90.0 a	2.4 a	48.6 a	53.2 b	334.0 b
				Non-nodulated				
R. japonicum	6.1 cd	8.5 b	51.6 b	0.0	0.0	0.0	0.0	0.0
R. solani + R. japonicum	4.4 ef	8.2 b	54.7 b	90.0 a	3.1 a	0.0	0.0	0.0
G. mosseae + R. japonicum	9.9 a	10.5 ab	56.0 ab	0.0	0.0	62.3 a	0.0	0.0
R. solani + G. mosseae + R. japonicum	7.0 bc	11.3 a	57.0 ab	100.0 a	2.5 a	44.2 b	0.0	0.0

[x] Inoculum densities were 2,000 sclerotia of R. solani/kilogram of soil; 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil), and 0.5% of R. japonicum (wt/wt) of the seeds.

[y] Disease index rating was based on the hypocotyl decay on a scale of 0 to 4 with 0 = no symptoms and 4 = most severe symptoms (dead plant).

[z] Values (means of 10 plants in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test), except for shoot weight.

number of nodules per plant (-0.51; P = 0.05), while the percentage of roots colonized with G. mosseae was positively correlated with mean root weight (0.46; P = 0.05) and mean nodule weight (0.44; P = 0.05) per plant.

Interaction among Fusarium solani, Glomus mosseae, and Rhizobium japonicum

There were no significant differences among non-nodulating and nodulating plants of the cultivar Hardee in all the parameters studied (Table 17). Inoculation with F. solani plus R. japonicum resulted in significantly lower root and shoot weight and inoculation with G. mosseae plus R. japonicum resulted in significantly higher root and plant height than in the other treatments. The mean root weights and shoot weights of nodulated and non-nodulated plants were decreased 54% and 56% by exposure to F. solani plus R. japonicum, respectively, as by compared with R. japonicum alone. Plant height of plants inoculated with F. solani plus R. japonicum was not significantly different from those plants inoculated with R. japonicum alone or F. solani plus G. mosseae plus R. japonicum; but plants inoculated with F. solani plus R. japonicum was significantly reduced from G. mosseae plus R. japonicum treatment. Glomus mosseae plus R. japonicum increased the mean plant height 30% in relation to R. japonicum alone.

Similar numbers of roots were infected by F. solani whether or not G. mosseae was present, but F. solani significantly reduced the percentage of roots colonized by G. mosseae in both nodulated and non-nodulated plants.

Fusarium solani in combination with R. japonicum significantly reduced number and weight of nodules per plant when compared to

Table 17. Soybean response, nodule number and weight, and percentage of roots infected with Fusarium solani and colonized by Glomus mosseae in combination with Rhizobium japonicum on nodulating and non-nodulating Hardee soybeans 45 days after infesting soil with known quantities of inoculum.[y]

Treatments	Root Weight (g)	Shoot Weight (g)	Plant Height (cm)	Roots with F. solani	Roots with G. mosseae	Nodules/plant Number	Nodules/plant Weight (mg)
			Nodulated				
R. japonicum	5.7 b[z]	5.4 a	37.0 bc	0.0	0.0	16.1 b	114.0 bc
F. solani + R. japonicum	2.2 c	3.2 b	33.0 c	48.0 a	0.0	10.3 b	20.0 c
G. mosseae + R. japonicum	8.1 a	6.4 a	53.5 a	0.0	49.9 a	30.0 a	393.0 a
F. solani + G. mosseae + R. japonicum	4.5 b	6.5 a	42.0 b	44.0 a	31.8 b	23.0 a	162.0 b
			Non-nodulated				
R. japonicum	4.7 b	5.8 a	34.0 c	0.0	0.0	0.0	0.0
F. solani + R. japonicum	2.6 c	3.1 b	37.0 bc	57.0 a	0.0	0.0	0.0
G. mosseae + R. japonicum	7.2 a	5,7 a	48.2 a	9.9	51,0 a	0.0	0.0
F. solani + G. mosseae + R. japonicum	4.9 b	5.8 a	42.0 b	53.0 a	30.5 b	0.0	0.0

[y] Inoculum densities were 3,000 x 10^3 chlamydospores of F. solani/kilogram of soil; 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil) and 0.5% of R. japonicum (wt/wt) of the seeds.

[z] Values (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test.)

Table 18. Concentration and amount of elements absorbed by nodulated and non-nodulated Hardee soybeans 45 days after infesting soil with known quantities of Rhizobium japonicum alone or in combination with Macrophomina phaseolina and Glomus mosseae.[y]

Treatments	Concentration of the elements in the shoot (ppm)								
	P	Ca	Mg	K	Zn	Cu	Fe	Mn	N
Nodulated	64.9 a[z]	236.6 a	106.8 a	587.0 a	2.95 a	0.17 a	3.08 a	6.40 a	3.41 a
Non-nodulated	69.2 a	239.8 a	108.3 a	546.5 a	2.85 a	0.17 a	2.48 a	6.80 a	2.69 b
	Amount of the elements absorbed by the shoot								
Nodulated	836.7 a	3108.4 a	1409.4 a	7690.3 a	38.0 a	2.29 a	42.1 a	79.8 a	44.2 a
Non-nodulated	719.0 a	2528.8 b	1129.8 b	5545.4 b	28.2 a	1.85 a	24.9 b	67.5 a	29.9 b

[y] Inoculum densities were 40 x 10^3 sclerotia of M. phaseolina/kilogram of soil, 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil), and 0.5% R. japonicum (wt/wt) of the seeds.

[z] Values (means of 40 plants) in vertical columns followed by the same letter are not significantly different (P<0.05 by Duncan's Multiple Range Test).

Table 19. Concentrations of elements absorbed by nodulated and non-nodulated Hardee soybeans 45 days after infesting soil with known quantities of Rhizobium japonicum alone or in combination with Macrophomina phaseolina and Glomus mosseae.[x]

Treatments	Concentrations of the elements in the shoot (ppm)								
	N[y]	P	Ca	Mg	K	Zn	Cu	Fe	Mn
					Nodulated				
R. japonicum	3.434 a	60.0 c[z]	206.8 a	107.4 a	504.0 bc	3.70 b	0.148 a	3.28 b	6.54 ab
M. phaseolina + R. japonicum	3.649 a	71.5 b	256.0 a	101.2 a	648.0 a	2.69 abc	0.156 a	2.20 b	7.96 a
G. mosseae + R. japonicum	3.007 abc	64.8 bc	253.0 a	114.6 a	584.0 abc	2.64 bc	0.186 a	3.42 a	5.46 b
G. mosseae + M. phaseolina + R. japonicum	3.552 a	61.7 c	230.8 a	104.2 a	612.0 abc	2.80 abc	0.190 a	3.44 a	5.64 b
					Non-nodulated				
R. japonicum	3.254 abc	65.0 bc	229.2 a	97.6 a	500.0 c	2.42 c	0.180 a	2.84 ab	8.16 a
M. phaseolina + R. japonicum	3.099 abc	64.0 bc	241.2 a	108.6 a	572.0 abc	3.06 abc	0.188 a	2.20 b	7.96 a
G. mosseae + R. japonicum	2.212 c	61.9 c	249.0 a	107.0 a	472.0 c	2.16 c	0.170 a	2.16 b	5.36 b
G. mosseae + M. phaseolina + R. japonicum	2.971 abc	85.9 a	240.0 a	119.6 a	642.0 ab	3.76 a	0.178 a	2.70 a	5.78 b

[x] Inoculum densities were 40 x 10^3 sclerotia of M. phaseolina/kg of soil; 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil), and 0.5% of R. japonicum (wt/wt) of the seeds.

[y] Value means percentage of nitrogen.

[z] Value (means of 10 plants) in vertical columns followed by the same letter are not significantly different ($P<0.05$ by Duncan's Multiple Range Test).

Table 20. Total amount of elements absorbed by nodulated and non-nodulated Hardee soybeans 45 days after infesting soil with known quantities of Rhizobium japonicum alone or in combination with Macrophomina phaseolina and Glomus mosseae.[x]

Treatments	Total amount of elements absorbed by the shoots (ppm)								
	N[y]	P	Ca	Mg	K	Zn	Cu	Fe	Mn
					Nodulated				
R. japonicum	39.97 ab	696.0 bcd[z]	2376.6 b	1231.7 bc	5748.2 bc	42.93 a	1.68 b	38.28 abc	75.45 ab
M. phaseolina + R. japonicum	30.97 bcd	600.6 bcd	2162.6 b	855.8 c	5467.0 bc	22.84 bc	1.31 b	18.62 cd	67.19 ab
G. mosseae + R. japonicum	50.99 a	1100.6 a	4314.9 a	1962.6 a	9874.6 a	44.61 a	3.14 a	57.61 a	93.16 a
G. mosseae + M. phaseolina + R. japonicum	55.48 a	955.0 ab	3579.5 a	1587.7 ab	9671.4 a	41.60 a	3.04 a	54.07 b	83.43 ab
					Non-nodulated				
R. japonicum	28.43 cd	587.7 cd	2070.3 b	892.7 c	4469.4 c	22.40 bc	1.64 b	24.69 cd	73.06 ab
M. phaseolina + R. japonicum	19.52 d	414.7 d	1547.9 b	697.4 c	3755.4 c	19.04 c	1.22 b	14.00 d	50.91 b
G. mosseae + R. japonicum	37.38 abc	1072.0 a	4241.7 a	1837.0 a	7996.4 ab	37.07 a	2.90 a	37.21 bc	93.74 a
G. mosseae + M. phaseolina + R. japonicum	38.02 abc	801.7 abc	2255.4 b	1091.9 bc	5960.4 bc	34.41 ab	1.64 b	23.74 cd	52.43 b

[x] Inoculum densities were: 40 x 10^3 sclerotia of M. phaseolina/kg of soil; 500 chlamydospores of G. mosseae/15 cm clay pot (2,000 g of soil), and 0.5% of R. japonicum (wt/wt) of the seeds.

[y] Value means percentage of nitrogen.

[a] Values (means of 10 plants) in vertical columns followed by the same letter are significantly different (P<0.05 by Duncan's Multiple Range Test).

G. mosseae plus R. japonicum; but treatment with F. solani and R. R. japonicum did not differ significantly from that with R. japonicum alone.

Nodule weight was reduced 82% by F. solani in combination with R. japonicum treatment alone. The number and weight of the nodules per plant were greater in the presence of G. mosseae plus R. japonicum with or without F. solani than with F. solani plus R. japonicum.

The precentage of roots colonized by G. mosseae was significantly correlated with the mean root weight (0.57, P = 0.05), plant height (0.47; P = 0.05), and number (0.70, P = 0.05), and weight (0.47, P = 0.05) of the nodules.

Discussion

Although growth of nodulated plants was greater than that of non-nodulated plants, differences were not significant in any of three experiments performed. This study, however, confirmed previous observations on the tripartite symbiosis among legumes, Rhizobium spp. and mycorrhizal fungi in which legumes in association with mycorrhizal fungi (Glomus spp.) have increased number and weight of nodules, total plant phosphate, root and shoot weight, and plant height over non-mycorrhizal plants (15, 23, 24, 25, 26, 84, 123).

Since growth of nodulated and non-nodulated plants did not differ significantly and G. mosseae also affected both equally, it appears that R. japonicum did not greatly influence growth response or infection by the pathogens in this study. For example, nodulated soybean plants inoculated with G. mosseae had 22% more total nitrogen than nodulated non-mycorrhizal plants, and non-nodulated plants inoculated

with G. mosseae had 24% higher total nitrogen than non-mycorrhizal plants. This failure of R. japonicum to affect the results may have been due to several factors. First, the nitrogen levels in the soil may have been too high for good Rhizobium development. Neither nodulated nor non-nodulated plants showed any foliar nitrogen deficiency symptoms and both nodulated and non-nodulated control treatments had similar concentrations of nitrogen in the shoots.

Secondly, the soil used in this study (serie Arredondo fine sand) might have had contents of organic matter with sufficient nitrogen for plant growth because it had been cropped with legumes successively for more than ten years. Similar seed yields to that obtained in this study for nodulated soybeans grown at high levels of nitrogen have been reported (53). Our results disagreed with those of Schenck and Hinson (108) in which mycorrhizal fungi increased soybean growth responses on the nodulated but not on the non-nodulated plants. Although the populations of soil-borne pathogens in the field used by Schenck and Hinson (108) were not reported, they postulated that soil-borne pathogens might have reduced growth responses on the non-nodulated cultivar, Hardee. Furthermore, different mycorrhizal species and soil nutrient levels were employed in the two studies. Not only are nodulation and nitrogen fixation by Rhizobium reduced by high concentration of nitrate, nitrite, ammonium and urea (25, 98), but these compounds induce high ammonium concentrations in roots which block the gene responsible for nitrogenase activity (129).

A significant reduction in root weight for both nodulated and non-nodulated plants occurred in the presence of M. phaseolina, R. solani and F. solani. It is probable that this root weight reduction

was associated with reduced plant growth due to the impairment of water and nutrient uptake resulting from root infection. These observations are consistent with reports of colonization of the vascular tissue by M. phaseolina and F. solani (16, 63), and root and stem rot by R. solani (12). Cowas (20) reported that infection of the roots of wheat seedlings by Gaeumannomyces graminis resulted in a considerable reduction in water consumption and reduced phosphorus levels in plant shoots when the supply of phosphorus to the seedlings was plentiful. Our results showed that plants infected with M. phaseolina had higher shoot concentrations of almost all elements than plants with R. japonicum alone, but both nodulated and non-nodulated plants with R. japonicum absorbed considerably higher total amounts of elements than plants with M. phaseolina plus R. japonicum. Plant growth response was inversely proportional to the concentration of elements in the shoots but directly proportional to the total amount absorbed by the plants.

When plants were exposed to combinations of M. phaseolina, R. solani, or F. solani plus G. mosseae and R. japonicum, soybean response was similar to that with R. japonicum alone in most cases. Since G. mosseae did not significantly affect the severity of the disease by the pathogens but did increase the level of nutrient absorbed from the soil, it is suggested that mycorrhizal plants can withstand better the attack of the pathogens by increasing nutrient uptake particularly of phosphorus, from the soil (49, 79, 80). In legumes, VA-mycorrhizal fungi not only stimulate plant growth but also promote nodulation and nitrogen fixation by Rhizobium (6, 23, 26, 84). Our results support the findings of Asai (4), Ross and Harper (103) and Daft and El-Giahmi

(24) which showed that uptake of two major elements, nitrogen and phosphorus, can be supplied partially to the host plant by means of symbiotic association. Significant stimulation of nodule numbers and weight by VA-mycorrhizal fungi was also confirmed in this investigation. This may have been due to increased accumulation of copper, phosphorus and zinc by mycorrhizal as compared to nonmycorrhizal plants (51, 103) since these elements have been reported to influence nodulation and nitrogen fixation (32, 33, 52, 58, 68, 80, 113).

Root colonization by G. mosseae was greatly influenced by the pathogens, whereas disease severity and root infection by the pathogens was not influenced by VA-mycorrhiza. Lower VA-mycorrhizal root colonization may be due to early colonization of readily infectable sites by the pathogens. The inoculum densities of the pathogens was greater than mycorrhizal inoculum and the pathogen inoculum was thoroughly mixed with the soil whereas the mycorrhizal inoculum was placed 5 cm below the seeds. Thus, the pathogens had the advantage over the mycorrhizal fungus for early infection and colonization. Reductions in percentages of colonization by mycorrhizal fungi by plant pathogens have been reported in other studies (27, 76). It appears that early colonization of the plant roots determined by the inoculum densities of mycorrhizal fungi and the pathogens is crucial in studying interactions between plant pathogens and VA-mycorrhizal fungi. Dehne and Schönbeck (30) for example reported that mycorrhizal tomato plants were less damaged at lower inoculum densities of F. oxysporum f. sp. lycopersici. The mycorrhizal effect was clearly reduced by an increase in inoculum density of the pathogen. Furthermore, prior root

colonization by VA-mycorrhizal fungi has been shown to greatly influence disease severity (29, 111).

Great reductions in nodule number and weight were caused by the three pathogens. Nodule weight reduction and a direct effect of R. solani on Rhizobium nodules has been reported (90). These findings were confirmed in this study, and F. solani was also observed to colonize soybean root nodules and reduce nodule number and weight.

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BIOGRAPHICAL SKETCH

Laércio Zambolim was born in Ubá, State of Minas Gerais, Brazil, on December 30, 1947. He received the degree of Agricultural Engineering at the Universidade Federal de Viçosa in 1970. In 1972, he received the Master of Science degree at the same university with a major in plant pathology. From 1973 to 1976 he worked for the Brazilian Enterprise (EMBRAPA/EPAMIG) at the Universidade Federal de Viçosa with chemical control of foliar diseases on bean, coffee and soybean.

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He was married to the former Eunize Maciel, December 27, 1975, and is the father of one child, Larissa.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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